THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

THE EFFECT OF FASTING UPON THE ACTIVITY OF SKELETAL MUSCLE. *C. E. Leese, H. M. Hines and D. P. Jordan* 241

THE EFFECT OF DISLOCATION OF CEREBROSPINAL FLUID UPON ITS PRESSURE. *Lewis H. Weed, Louis B. Flexner and Janet H. Clark* 246

HETEROTOPIC BONE FORMATION IN THYROPARATHYROIDECTOMIZED DOGS. *Carl A. Dragstedt and John E. Kearns, Jr.* 262

THE SPECIFICITY OF PANCREATIC LIPASE: ITS APPEARANCE IN THE BLOOD AFTER PANCREATIC INJURY. *Ian S. Cherry and Lathan A. Crandall, Jr.* 266

METABOLISM IN YUCATAN: A STUDY OF THE MAYA INDIAN. *Morris Steggerda and Francis G. Benedict* 274

INSULIN IN TISSUES OTHER THAN THE PANCREAS. *C. H. Best, C. M. Jephcott and D. A. Scott* 285

THE EFFECTS OF SYMPATHETIC DENERVATION OF MAMMALIAN TISSUES DURING THE PERIOD OF POST-NATAL GROWTH. *S. S. Tower* 295

THE EXCRETION OF URINE IN THE DOG. III. THE USE OF NON-METABOLIZED SUGARS IN THE MEASUREMENT OF THE GLOMERULAR FILTRATE. *Norman Jolliffe, James A. Shannon and Homer W. Smith* 301

ACTION OF EPINEPHRINE ON THE INTESTINE FOLLOWING STIMULATION BY PARASYMPATHETIC DRUGS. *Frederick Bernheim and Berget H. Blocksom, Jr.* 313

STUDIES OF GALL-BLADDER FUNCTION. VII. THE ANION-CATION CONTENT OF HEPATIC AND GALL-BLADDER BILE. *I. S. Ravdin, C. G. Johnston, C. Riegel and S. L. Wright, Jr.* 317

SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING. IX. URIC AND LACTIC ACIDS. *A. K. Saiki, G. Olmanson and G. A. Talbert* 328

A STUDY OF THE EXCISED UTERUS OF THE RAT, ITS VOLUME DISPLACEMENT AND IRRITABILITY TO PITUITRIN, WITH REFERENCE TO THE OESTROUS CYCLE. *O. G. Harne* 331

STUDIES ON THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN). XI. EFFECT OF CROTALIN ON SWINE. *Hiram E. Essex* 339

THE FAILURE OF DIET TO INFLUENCE THE ADRENIN CONTENT OF THE ADRENAL GLANDS. *Arturo Rosenblueth and René Gayet* 342

THE CURRENT FLOWING THROUGH THE HEART UNDER CONDITIONS OF ELECTRIC SHOCK. *William B. Kouwenhoven, Donald R. Hooker and Orthello R. Langworthy* 344

THE DIGESTIVE LEUCOCYTOSIS QUESTION. *W. E. Garrey and Virginia Butler* 351

THE EFFECT OF CAFFEINE ON THE PREVAGUS AND POSTVAGUS CHICK HEARTS. *Floyd J. Brinley* 357

STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE. III. THE LONGITUDINAL AND CIRCULAR ACTIVITY OF THE DISTAL COLON. *Hampden Lawson and R. D. Templeton* 362

AN ANALYSIS OF THE RHYTHMIC VASCULAR CHANGES IN THE UTERUS OF THE RABBIT. *J. E. Markee* 374

CARDIOVASCULAR RESPONSE OF HEALTHY YOUNG MEN TO POSTURAL VARIATIONS AT VARIED TEMPERATURES. *Joseph E. Hamilton, Joseph S. Lichty and William R. Pitts* 383

THE HYDROGEN ION CONCENTRATION OF THE GASTRIC JUICE OF FETAL AND NEWBORN WHITE RATS. *Ira A. Manville and Robert W. Lloyd* 394

A COMPARISON OF THE RELATION BETWEEN THE RATE OF UREA EXCRETION AND THE AMOUNT OF RENAL TISSUE IN THE DOG AND OTHER MAMMALS. *Eaton M. MacKay* 402

CAPILLARY COUNTS IN RESTING AND ACTIVE MUSCLE. *E. G. Martin, Eola C. Woolley and Miriam Miller* 407

(Continued on cover 4)

VOL. C—NO. 2

Issued April 1, 1932

BALTIMORE, U. S. A.

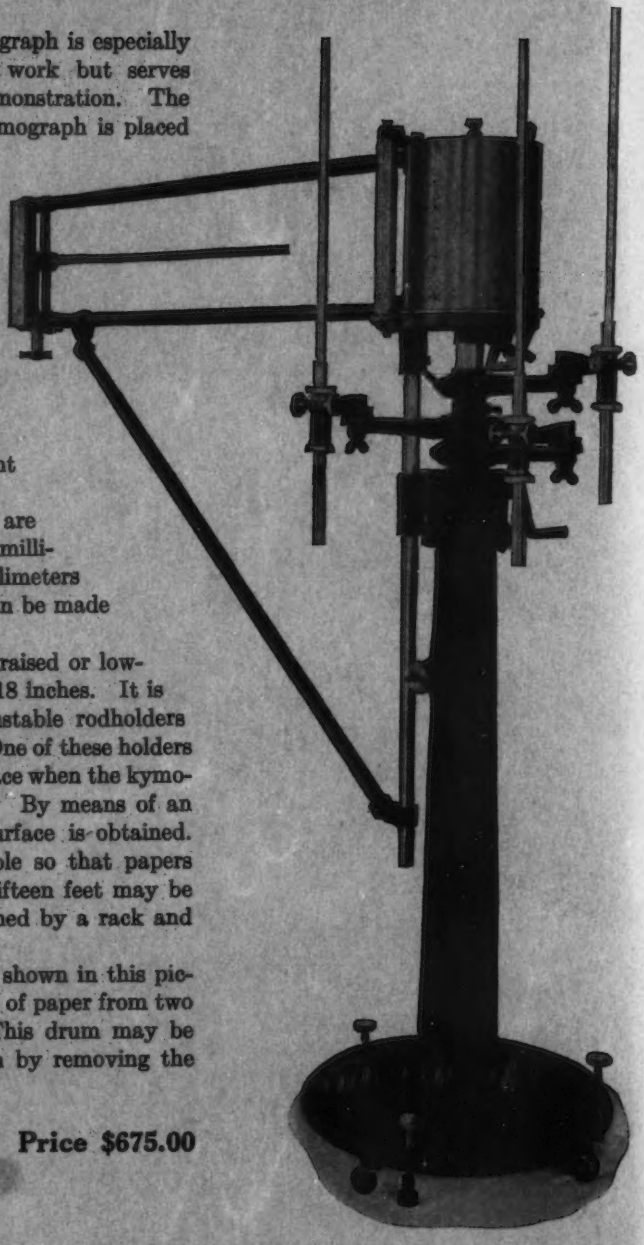
1932

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

Made in United States of America

LONG PAPER KYMOGRAPH

THIS forty speed Kymograph is especially designed for research work but serves admirably for student demonstration. The motor which drives the kymograph is placed within the large drum, thus doing away with all troublesome belts and making it possible to mount the complete kymograph on a pedestal, the base of which is 20 inches in diameter and is equipped with castors and leveling screws so that the apparatus may be moved to any convenient place.



Forty changes of speed are available, ranging from 50 millimeters per hour to 110 millimeters per second. All changes can be made rapidly.

The kymograph may be raised or lowered through a distance of 18 inches. It is equipped with three adjustable rodholders and fine adjusting screw. One of these holders moves with the writing surface when the kymograph is raised or lowered. By means of an idle drum a flat writing surface is obtained. The rear drum is adjustable so that papers varying between eight to fifteen feet may be used. The paper is tightened by a rack and pinion.

An additional drum, not shown in this picture, is provided for the use of paper from two to three feet in length. This drum may be adjusted on the upper arm by removing the adjustable rodholder.

Price $675.00

JOSEPH BECKER

630 West 168th Street, **NEW YORK CITY**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 100 APRIL 1, 1932 No. 2

THE EFFECT OF FASTING UPON THE ACTIVITY OF SKELETAL MUSCLE[1]

C. E. LEESE, H. M. HINES AND D. P. JORDAN

From the Department of Physiology and the Child Welfare Station, State University of Iowa

Received for publication December 28, 1931

The purpose of this investigation was to determine the effect of varying periods of fasting upon the activity of the intact skeletal muscle of the rat. The phases of activity studied were the number of artificial stimuli required to produce fatigue and the amount of work accomplished by the muscle during the total period of activity. Studies were made upon the glycogen content of muscle and liver from the exercised and control animals in order to determine whether a relationship existed between the amount of glycogen stores and muscular activity.

PROCEDURE. Adult rats selected from the general laboratory stock were weighed and placed in separate cages and subjected to fasting for periods varying from 24 to 144 hours in duration. Water ad libitum was allowed throughout the fasting period. The animals designated as non-fasting were taken directly from the stock cages which contained at all times an abundant diet of soy beans, corn, dog biscuits, milk and greens. A satisfactory degree of surgical anesthesia was produced by the intraperitoneal injection of 6 mgm. of amytal per 100 grams of body weight. Additional amounts of anesthetic were administered from time to time as needed. The extent of the superficial reflexes was used as a criterion of the depth of anesthesia. Control and experimental animals were subjected to the same depth and duration of anesthesia.

The gastrocnemius muscle was exercised by maximal faradic break shocks under conditions which permitted the utilization of its natural attachments, thus avoiding as far as possible disturbances due to distortion or overstretching. The leg was fixed in a rigid position by a modified femur clamp attached to the tibia. A cord was fastened to the foot by a pin hook which was thrust through the middle phalangeal joints

[1] Aided by a grant from the Laura Spelman Memorial Fund.

of the toes. This cord was attached to a muscle lever weighted with a load equal to one half of the animal's weight. Flexible glass shielded electrodes were attached to the tendon achilles and gastrocnemius muscle. A motor driven device equipped with a cyclometer was employed to deliver supermaximal break induction shocks to the muscle at the rate of 2 to 3 per second. The muscular contractions were recorded on a slow moving extension kymograph. The muscle was considered to be fatigued when there occurred no perceptible movement of the lever. The amount of work done was calculated from the load lifted and the height of the contractions. Corrections were made for the magnification due to lever-

TABLE 1

A summary of the effects of activity and fasting upon muscle and liver glycogen

(Mgm. per 100 grams of tissue)

	LIVER		MUSCLE	
	Control	Exercised	Control	Exercised
Non-fast	2,185	164	537	125
P.E.M.	±585	±23	±61	±16
24 hr. fast	172	67	370	74
P.E.M.	±15	±15	±8	±9
48 hr. fast	78	58	375	56
P.E.M.	±19	±9	±20	±11
80 hr. fast	254	72	356	90
P.E.M.	±29	±9	±7	±12
120 hr. fast	345	46	321	109
P.E.M.	±49	±7	±11	±8
144 hr. fast	339	84	355	131
P.E.M.	±70	±16	±14	±7

age. At the conclusion of the experiment the gastrocnemius muscle and liver were quickly excised, weighed and placed in hot 60 per cent NaOH. The glycogen was determined according to the method of Pflüger as modified by Bollman, Mann and Magath (1925). The sugar content of the hydrolyzed glycogen was determined by the method of Shaffer and Hartmann (1920). In order to learn something as to the general condition of the animal, determinations of the lactic acid content of heart's blood were made at the end of some of the experiments.

RESULTS. The mean values for the glycogen content of liver and muscle from the fatigue and control rats are presented in table 1. They represent an average of determinations made on eight or more animals

in each group. The values for the probable error of the mean are presented to indicate something as to the variations in individual experiments. It was found that a fast of 24 hours materially reduced the glycogen content of muscle. Longer periods of fasting did not bring about any further reduction in muscle glycogen. Fasting markedly decreased the glycogen content of the liver. The lowest values were found at the end of a 48 hour period. Apparently liver glycogen diminished rapidly in the early hours of fasting and then increased during the longer periods to values well above the minimal. Exercise caused a great reduction in muscle and liver glycogen. However, demonstrable quanti-

TABLE 2

A summary of the effect of fasting upon body weight and activity of intact gastrocnemius muscle

	NON-FAST	24 HR. FAST	48 HR. FAST	80 HR. FAST	120 HR. FAST	144 HR. FAST
Per cent loss of body weight		6.0	9.5	16.9	20.9	22.5
Stimuli to fatigue in thousands	67	71	67	95	69	116
Probable error of mean	±6	±10	±10	±10	±6	±9
Time in hours to fatigue	6.7	7.3	6.7	11.4	7.9	12.0
Probable error of mean	±0.6	±0.9	±1.0	±1.6	±0.6	±1.1
Total work* per gram of muscle	2.8	2.7	2.8	3.2	2.0	3.1
Probable error of mean	±0.3	±0.4	±0.4	±0.5	±0.2	±0.6
Work* per gram of muscle during 1st 1000 contractions	0.26	0.24	0.28	0.27	0.18	0.22
Probable error of mean	±0.02	±0.02	±0.01	±0.02	±0.02	±0.03
Work* per kgm. of original body weight	13.4	11.8	12.0	15.3	9.5	15.1
Probable error of mean	±1.9	±1.4	±1.6	±2.6	±0.9	±2.7

* Work in kilogram-meters.

ties of glycogen were present in the muscle and liver at a time when the muscle was considered to be in a state of complete fatigue. No relationship appeared to exist between the time required to fatigue or the amount of work performed and the quantity of muscle and liver glycogen present before and after fatigue. Although considerable variation existed in the individual experiments, it was apparent that fasting did not affect the amount of work done by a muscle or the number of stimuli required to produce fatigue (table 2). The amount of work done by the muscle has been expressed in terms of the number of kilogram meters per gram of gastrocnemius muscle, kilogram meters per kilogram of body weight

before the beginning of the fast, and the amount of work accomplished during the first 1000 contractions. Muscles from the 120 hour fasting group exhibited the least susceptibility to fatigue as judged by the time required and the amount of work performed during activity. The average values for the lactic acid content of heart's blood from the fatigued animals was approximately twice that found in the anesthetized controls.

Discussion. The work on the effects of fasting upon the activity of man and animals is entirely too voluminous to be discussed here. Readers are referred to Morgulis (1923) for an excellent account of many such experiments. In general, the work of previous investigators has shown that fasting and under-nutrition are accompanied by diminished ability to do work and an increased susceptibility to fatigue. A few instances are recorded in which an individual's strength was apparently increased for a short time during a fast and in which isolated muscles from fasting animals showed a diminished rate of fatigue. Many of these observations were made under experimental conditions which did not permit one to draw conclusions as to whether the effects of fasting upon activity were related to alterations in the stimulus or the ability of the muscle to respond. In our work we have attempted to control the former factor by applying directly to intact muscle electrical stimuli of strengths greater than that required to produce maximal contractions. It is believed that the preservation of the natural muscular attachments permits one to approach more nearly the true physiological conditions of the natural activity of muscle. It was our experience that this arrangement permitted a muscle to do much more work than when the cut tendon method was employed. We believe the lessened activity found when the latter method was employed to be due to an unavoidable interference with the circulation in the muscle due to its distortion or overstretching. The values found for the lactic acid content of heart's blood at the end of the experiments indicate that the failure of the muscle to exhibit further contractions was not due to a collapse of the systemic circulation and respiration. The total amount of work done by kilogram of original body weight is regarded as the most reliable of the various criteria of activity employed (table 2). The total number of stimuli and the total time required to produce fatigue are regarded as less significant because of the difficulty of determining the exact time at which the muscle no longer exhibited perceptible shortening. In some experiments the muscles exhibited a very slight degree of shortening for several hours. In other experiments the duration of this period of slight activity was much shorter.

It is apparent from the results of these experiments that the skeletal muscle from animals that had been fasted for periods as long as 144 hours was able to perform as much work as muscle from control non-fasted animals. The mean values observed in the experiments on the 144 hour

group are suggestive of an increased efficiency during fasting. However, when one considers the range of variation in the individual experiments and the fact that the activity of the 80 hour fasted group was slightly less than that of the controls, it is apparent that this suggestion cannot be regarded as significant. No studies were made on animals fasted for periods longer than 144 hours. We wished to avoid, as far as possible, the complications that might arise from the use of anesthesia for long periods in the terminal stages of fasting. The amount of work performed and the rate of fatigue were found to be independent of the amounts of glycogen contained in the liver and muscle at the beginning and at the end of the experiments. Muscles from animals with glycogen poor livers exhibited as much activity as muscles from animals having a normal glycogen content. At the conclusion of the experiment demonstrable quantities of glycogen were found to be present in the liver and exercised muscle of all animals. There appeared to be no relationship between the amount of work done by a muscle and the amount of glycogen depletion in the liver and muscle. Our findings are in harmony with the observations of Witting, Markowitz and Mann (1930) who found the survival time of perfused glycogen poor hearts to be as long as that of hearts with normal amounts of glycogen.

SUMMARY

A study has been made of the effects of fasting upon the activity of the artificially stimulated intact gastrocnemius muscle of the anesthetized rat. The phases of activity studied were the number of stimuli required to produce fatigue and the amount of work done by the muscle. The results indicate that fasting for periods of 24 to 144 hours did not affect either the rate of fatigue or the total amount of work done by the artificially stimulated intact muscle. Studies were made upon the glycogen content of liver and muscle from control and exercised animals. In all experiments, fatigue occurred before an exhaustion of liver and muscle glycogen. The amount of work performed by the muscle and the rate of fatigue were found to be independent of the amount of glycogen present in liver and muscle at the beginning and at the end of the experiments.

BIBLIOGRAPHY

Bollman, J. L., F. C. Mann and T. B. Magath. 1925. This Journal, lxxiv, 238.
Morgulis, S. 1923. Fasting and under-nutrition. New York.
Shaffer, P. A. and A. F. Hartmann. 1920. Journal Biol. Chem., xlv, 365.
Witting, V., J. Markowitz and F. C. Mann. 1930. This Journal, xciv, 35.

THE EFFECT OF DISLOCATION OF CEREBROSPINAL FLUID UPON ITS PRESSURE

LEWIS H. WEED, LOUIS B. FLEXNER AND JANET H. CLARK

From the Department of Anatomy, Johns Hopkins University

Received for publication January 8, 1932

In the interpretation of the changes in the pressure of the cerebrospinal fluid, caused by tilting a four-footed animal from the horizontal to the vertical positions, stress was necessarily laid upon the possible dislocation of the fluid from the uppermost portions of the nervous system into the dependent (Weed, 1929b). The measurements of the pressure of the cerebrospinal fluid were made, in this series of experiments, by means of the customary open-end manometer of small bore, slightly over 1 mm. in diameter. The pressures obtained by this method have always been taken to be a fairly accurate record of those existing within the subarachnoid space; but there are of course certain hypothetical objections to the use of a system of such small bore, particularly when the fluid in the manometer is normal saline solution or cerebrospinal fluid. In the 1 mm. system, filled with these fluids, the theoretically upward pull of the fluid, due to capillary forces, has been assumed to be of no significance, as the continued pulsations of the fluid constantly alter the surface tension of the meniscus and thereby eliminate any capillary pull.

In the customary group of experimental conditions necessitating measurement of the pressure of the cerebrospinal fluid, the open-end manometer of small bore has, because of its simplicity, proven eminently satisfactory. It has always been realized that such an open-end manometer, either as a straight apparatus or as a U-shaped tube for measuring negative pressures, introduces the element of atmospheric pressure more or less directly upon the central nervous system. For under the conditions of measurement of the fluid-pressure, the meniscus of fluid in the manometer must be taken as indicating the level of atmospheric pressure. While in most experiments upon the cerebrospinal fluid the readings in the open-end manometer will serve as an accurate guide to pressure-changes within the subarachnoid space, they cannot be assumed to be as exact as those obtained in a system which permits measurement without dislocation of fluid. The most satisfactory method of determining fluid-pressures without dislocation is that of the so-called "bubble manometer" in which a bubble of air is confined within a glass tube of small bore. The pressures

are read by the height of the column of fluid needed to maintain the bubble in an arbitrarily established position, constituting zero (or atmospheric) pressure when the distal end of the system beyond the bubble is filled with fluid and open to the atmosphere. Any increase or decrease in this pressure, on connection to the animal, is represented by a dislocation of the imprisoned air-bubble; adjustment of the reservoir restores the bubble to the previous position, so that determination of the pressure imparted by the reservoir of fluid gives the record of the pressures within the animal without dislocation of fluid.

While it has proven true that pressures of the cerebrospinal fluid may be obtained with a fair degree of accuracy in manometers of small bore and of open end, certain types of experiments have aroused question as to the employment of this method in those cases where great differences in the pressures recorded involve the displacement of relatively large amounts of fluid. Our own attention was acutely called to this problem by the great pressure-alterations obtained in the cerebrospinal fluid on abrupt tiltings of the animal from the horizontal to the vertical head-down and tail-down positions (Weed, 1929a, b; Weed and Flexner, 1932). In these experiments pressure-changes of 200 mm. were not uncommon. As such changes involved a considerable dislocation of fluid, even with a 1 mm. manometer, it was thought that a comparison of the findings with different manometers in living animals and in artificial systems might be of interest. The results brought out a definite and important relationship between the dislocation of cerebrospinal fluid and its pressure.

Methods of investigation. A series of small dogs of fairly uniform size (weight, 6–7 kgm.) was employed for the experiments. The animals were all given ether and attached to a tilting board. The customary puncture into the subarachnoid space through the occipito-atlantoid ligament was made on each animal and the needle connected to manometers of different bores. In the usual experiment the manometer, initially attached, was a modification of the bubble manometer, in which a single bubble of air was imprisoned in a long glass tube of approximately 1 mm. bore. The initial readings with the bubble manometer were followed by connection of open-end manometers of 1 mm., 2 mm., 4 mm., 6 mm., 8 mm., 10 mm., and in some experiments 16 mm., 26 mm., and 45 mm. bore.

In each instance, with the attachment of the different manometers, the fluid pressure was restored to approximately the pre-existing level for the animal in horizontal position. The animals were then tilted to the vertical positions, head-down and tail-down, and readings of the pressure of the cerebrospinal fluid taken. At the conclusion of the experiments the animals were killed.

In addition to the observations made on living animals a series of similar experiments was carried out on a system of glass tubes and tam-

bours, which simulated conditions in the living animal. This artificial system consisted of a straight glass tube, 400 mm. in length (corresponding to the length of the spinal column in the dogs used). Attached to this tube was a cross-bar for connection of the manometer and an extension of a 70 mm. tube, representing the head-end of the dog beyond the needle. The system was so arranged that tambours of different diameter, covered with rubber membranes, could be attached at either end. The artificial apparatus was filled with fluid after connection of manometers, and positive pressures of the desired degree obtained from a fluid reservoir. The elastic membrane of the tambour permitted the maintenance of these positive pressures by distending the membrane. The whole glass tube-tambour system was attached to the tilting table so that readings could be taken in a horizontal position and subsequently in positions corresponding to the vertical head-down and tail-down positions of the experimental animal. With possible dislocation of fluid into the open-end manometer or into the dependent tambour, a series of observations of the pressure-changes was made with the various manometers used on the experimental animal.

EXPERIMENTAL FINDINGS. With the series of manometers graded both by bore and by capacity, the two series of observations—one on the living animal and one on the artificial system,—were carried out. The results of these two types of experiments are given below.

Experimental animals. In the anesthetized dog, the bubble manometer gave readings of the pressure of the cerebrospinal fluid in the horizontal position of the same magnitude as with an open-end, small-bore manometer. This pressure was usually in the neighborhood of 125 mm. of normal saline solution, but due to differences in age and some individual variation, these horizontal pressures varied somewhat from this mean. The pressure-alterations in the cerebrospinal fluid on tilting, as determined by the closed manometer, were slightly greater than with the ordinary 1 mm. manometer, but these differences were so slight as to call attention to the general accuracy of all readings obtained by the open-end 1 mm. manometer, as customarily employed in determinations of the pressure of the cerebrospinal fluid. With the larger manometers, all of the pressure-alterations varied in accordance with the bore of the manometer, the larger manometers giving much smaller differences in the pressure-changes on tilting from the horizontal to the vertical positions.

These generalizations are best illustrated by the record of a single animal, in which a series of observations was made with manometers of different bore and with the bubble manometer attached at the beginning and the end of the experiment. Chart 1 shows such a record, carried out continuously on an etherized dog. The pressures have been arbitrarily brought back to a common base line, about 200 mm. positive pressure,

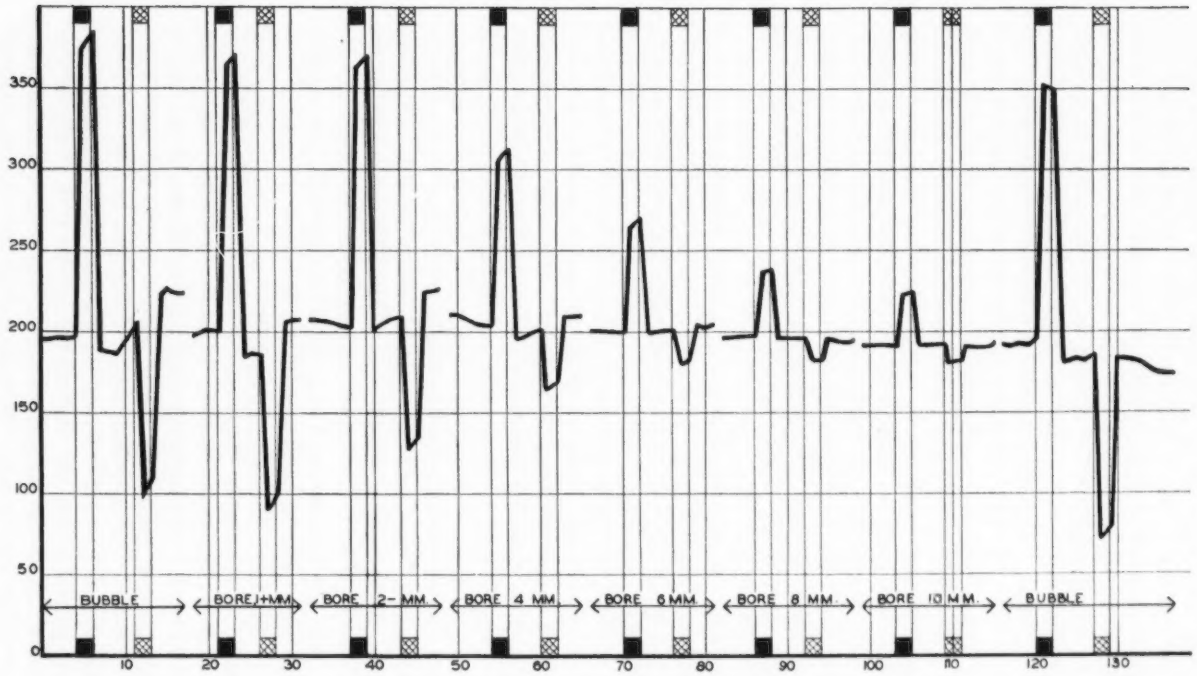


Chart 1 (dog, expt. A-100). The ordinates represent millimeters of normal saline solution; the abscissae, time in minutes. The chart records the pressure of the cerebrospinal fluid from an occipital manometer. The animal was in the horizontal position except during intervals marked by the solid blocks when it was tilted to the vertical head-down position, and during intervals marked by the cross-hatchings when it was tilted to the vertical tail-down position. As indicated on the chart, the pressure of the cerebrospinal fluid was recorded by bubble manometer and by open-end manometers of various bores (from 1 mm. to 10 mm.).

so that ease of comparison of the magnitude of the alterations could be attained. In the first period of this experiment the bubble manometer was used, permitting observations of pressure without dislocation of fluid. Here shift from the horizontal to the vertical head-down position resulted in an increase of pressure of 177 mm. in the cerebrospinal fluid, while the tilt from the horizontal to the tail-down position gave a pressure-reduction of 112 mm. With the substitution of the manometer of approximately 1 mm. bore the vertical head-down tilt gave a pressure-increase of 167 mm., while the vertical tail-down shift showed a decrease of 94 mm. As progressively larger manometers were used, the pressure-changes in the cerebrospinal fluid on tilting to the two vertical positions became smaller and smaller, as shown in chart 1. After this series with open-end manometers was completed, the bubble manometer was again attached; and on tilting from the horizontal to the vertical head-down position a pressure-increase of 157 mm. was noted, while on tilting from the horizontal to the vertical tail-down position a decrease of 111 mm. was recorded.

In the particular experiment recorded in chart 1, no use was made of manometers larger than 10 mm. bore. When these larger manometers were used, the results were quite in accord with the series illustrated in chart 1. Thus in experiment A-95, the pressure of the cerebrospinal fluid when recorded by the bubble manometer, rose 135 mm. on tilting to the vertical head-down position, and fell 79 mm. on the opposite vertical tilt. With a manometer of 26 mm. bore, the increase in pressure of the cerebrospinal fluid, on the vertical head-down tilt, was noted to be 4 mm., while the decrease in pressure on tilting from the horizontal to the vertical tail-down position was 2 mm. The difficulty of accurate reading, with such small changes in the height of the fluid level and with the large meniscus of fluid, was even more strikingly brought out in the attachment of a manometer of 45 mm. caliber. Because the determination of the level in a manometer of such great bore could not be accurately made, the readings were not continued, although they showed results which were quite in keeping with the decreasing pressure-alterations noted in the larger and larger manometers.

Consideration of the results obtained with the open-end manometers of different bore in comparison with the pressure-alterations noted in the bubble manometer, led us to speculate regarding the relation of dislocation of fluid to the pressure recorded. It was realized that as the bore of the manometer increased a greater and greater dislocation of fluid from the central nervous system into the manometer was necessary to achieve any given pressure-alteration on the vertical head-down tilts, while on the vertical tail-down shifts the dislocation of fluid was from the manometer into the central nervous system. It was obvious that the base-line of

pressure-alteration was necessarily that attained by the bubble manometer, for in this case no dislocation of fluid into or from the manometer system could be assumed, and such alterations in pressure as were measured by the bubble manometer represented true pressure-alterations without dislocation of fluid into or from any external system.

In a series of experiments the manometers were all calibrated as to the volume of fluid dislocated in the pressure-alterations of the magnitude obtained. The determination of the capacity of the manometers was made in two ways and the results checked against each other. By the first of these methods actual weight of mercury contained in the manometer in the pressure-change noted was determined; and in the second method, a theoretical computation of the weight of the fluid made. The two determinations checked on some of the manometers within a fairly close range, indicating that these manometers were of fairly accurate bore throughout; others of the manometers showed far greater inequalities in caliber.

It was found that these determinations of the volume of displaced fluid had a fairly constant relationship to the pressure-changes obtained in any one animal. This relationship was expressed in a fraction or quotient designated as $\frac{dV}{dP}$, where dV represents the difference in volume between the experiment with no displacement (bubble manometer) and the actual cubic centimeter change in any of the open-end manometers, while dP represents the difference in centimeters of the pressure-change on tilting, between that recorded by the bubble manometer and those by any of the open-end manometers. Calculating this fraction, $\frac{dV}{dP}$, from the results of the tiltings to the vertical head-down and tail-down positions a fairly constant value was obtained in most of the experiments. This is shown in table 1, which represents the data from two typical experiments, both carried out on dogs of the more or less standard size and weight used in these observations. In the whole series, the fraction $\frac{dV}{dP}$ was found to have an average value of 0.17. Calculations of the value of the fraction $\frac{dV}{dP}$ may be also made by using the pressure-changes from any two of the open-end manometers with their volume-differences; and by this method of determination similar values are obtained.

Consideration of these findings led to the generalization that the coverings of the central nervous system and cerebrospinal fluid may be likened to a container of unknown volume with elastic elements of an unknown coefficient of elasticity. This encasement approximates a rigid container because of the low elasticity of the walls and the character of the enclosing

bony sheath. According to such a conception it becomes possible to consider the application of the physical laws of an elastic system. The general expression for any coefficient of elasticity is the quotient obtained by dividing the stress by the strain. In the coefficient of volume-elasticity E, the stress is the change in pressure dP and the strain is the change in volume dV divided by the original volume V. Or, E = coefficient of elasticity $= dP/\frac{dV}{V} = \frac{dP}{dV} V$.

In the central nervous system, the volume-elasticity of the membranes leads to a dislocation of fluid, amounting to the volume dV, which is

TABLE 1

Table showing the derivation of the fraction $\frac{dV}{dP}$ *from two dogs (A-95 and 99), giving relationship between the pressure of the cerebrospinal fluid and the volume of fluid dislocated*

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN				EXP. NO.
	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*		
Bubble	14.8	0	0		3.8	0	0		A-99
1 mm.	13.3	1.5	0.231	0.154	3.4	0.4	0.059	0.148	
4 mm.	8.1	6.7	0.879	0.131	2.3	1.5	0.228	0.152	
8 mm.	3.3	11.5	1.68	0.146	0.9	2.9	0.458	0.158	
26 mm.	0.4	14.4	2.58	0.179	0.1	3.7	0.645	0.174	
Bubble	13.5	0	0		7.9	0	0		A-95
1 mm.	12.0	1.5	0.192	0.128	7.3	0.6	0.116	0.193	
4 mm.	7.1	6.4	0.770	0.120	5.5	2.4	0.480	0.200	
8 mm.	2.8	10.7	1.43	0.134	1.5	6.4	0.900	0.141	
26 mm.	0.4	13.1	2.58	0.197	0.2	7.7	1.29	0.167	

related to a change in pressure dP. The ratio $\frac{dV}{dP}$, derived above from our experiments, may be considered to be equal to $\frac{V}{E}$ where V is the original volume of the fluid-system (intradural contents) and where E is the coefficient of elasticity of the physiological system which here is a complex function involving vascular tone as well as membrane elasticity. In any one animal, the normal volume V does not seem to vary appreciably as any theoretical or actual dislocation of cerebrospinal fluid may be entirely or partially compensated by a reciprocal readjustment within the vascular channels. Assuming then that the volume V in any one

animal has a normal value within the limits of this reciprocal vascular readjustment, one would expect the fraction $\frac{dV}{dP}$ to remain constant as long as the animal remained in good condition with normal responses. In most of our experiments this was found to hold true. There were some animals however in which the fraction was different for the head-down and tail-down tilt. As V was the same, this difference indicated an abnormality in the physiological response to positional change. The most extreme variations in any one animal were between the limits of 0.048 (tail-down tilt) and 0.223 (head-down tilt). In this particular animal the pressure-changes on head-down tiltings gave a fair degree of

TABLE 2

Table showing derivation of the fraction $\frac{dV}{dP}$ in a dog (A-100) on vertical head-down and tail-down tiltings with manometers of various calibers

The pressure-change set down for the bubble manometer, 17.9 cm., represents the average of the first two tiltings (177 mm. and 181 mm.) on head-down tiltings while the corresponding figure for tail-down tiltings 11.2 cm., is the first of the two readings (112 mm. and 111 mm.).

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN			
	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*	
Bubble	17.9	0	0		11.2	0	0	
1 mm.	16.7	1.2	0.267	0.223	9.4	1.8	0.150	0.083
2 mm.	16.0	1.9	0.290	0.152	8.3	2.9	0.150	0.051
4 mm.	10.2	7.7	1.012	0.131	3.7	7.5	0.367	0.049
6 mm.	6.7	11.2	1.77	0.158	1.7	9.5	0.46	0.048
8 mm.	3.8	14.1	1.934	0.137	1.1	10.1	0.558	0.056
10 mm.	3.2	14.7	2.28	0.155	0.9	10.3	0.638	0.062

constancy to the fraction as did also the pressure-changes on tail-down tiltings (table 2), indicating a marked difference in the elasticity of the system in these two vertical positions. Minor alterations in elasticity occurred in many experiments, resulting in slightly variable reactions to tiltings. Thus in this same animal (A-100), the pressure-increases obtained with the bubble-manometer on tilting from the horizontal to the vertical head-down position were 177, 181, and after an interval, 157, 161, 174, 168 mm., on observations throughout the period of experimentation; the pressure-decreases on tail-down tiltings were 112 and 111 mm. Under these circumstances it seemed necessary to determine the magnitude of the pressure-alterations with the bubble-manometer as a series,

taking the average of the alterations rather than a single observation for the establishment of the base-line of reference. This inconstancy of results in positional pressure-alteration is graphically shown in chart 4 of a previous report (Weed, 1929b), in which repeated tilting to the tail-down position was practiced, and may be interpreted as due to minor variations in the physiological elasticity of the system.

Provided no marked alteration in elasticity takes place throughout an experiment, resulting in a constant value for the fraction $\frac{dV}{dP}$, it should be possible to calculate the change in volume of the intradural contents from a recorded change in pressure, and in this way to determine the amount of fluid abstracted from the nervous system by the intravenous injection of a strongly hypertonic solution (vid. Weed and McKibben, 1919a, b; Weed and Hughson, 1921a, b, c, et al.). In other words, the amount of brain shrinkage and loss of cerebrospinal fluid, uncompensated by reciprocal dilatation of the vascular bed (vid. Weil, Zeiss and Cleveland, 1931), might be computed by multiplying the pressure-change in the cerebrospinal fluid by the factor $\frac{dV}{dP}$, thus determining the unknown volume-change within the membranes of the central nervous system.

To this end a series of experiments on etherized dogs of the same size was undertaken. Pressures of the cerebrospinal fluid were determined in the horizontal position throughout by attachment of a U-manometer (1 mm. bore, capable of recording negative pressures), to a needle inserted into the subarachnoid space through the occipito-atlantoid ligament. The animal (B-1), after an initial period of control, was given intravenously 20 cc. of 30 per cent solution of NaCl and subsequently an additional intravenous injection of 10 cc. of the same solution. The pressure of the cerebrospinal fluid dropped rapidly, and 25 minutes after the completion of the intravenous injection a negative pressure, hovering about minus 150 mm., was attained. The total alteration in the pressure of the cerebrospinal fluid was at this time 23.0 cm. (from plus 80 mm. to minus 150 mm.). If this pressure-alteration be multiplied by the quotient (0.17), the product 3.91 should indicate in cubic centimeters the actual diminution in the intradural contents, effected by the hypertonic solution. To test experimentally this computation, a very small puncture hole was made through the calvarium and cranial dura, and a bland mineral oil in the amount of 4.4 cc. was injected into the cerebral subarachnoid space from a finely calibrated syringe. The pressure of the cerebrospinal fluid rose to plus 90 mm.—10 mm. above the initial reading in this animal. Multiplying this pressure-difference (24.0 cm.—from minus 150 mm. to plus 90 mm.) by the factor 0.17, gives a product of 4.08 cc., to be contrasted with the actual injection of 4.4 cc. The difference between the

actual and computed values here is decreased when correction of 0.37 cc. is made for the fluid displaced into the manometer during the rise from minus 150 mm. to plus 90 mm.

Subsequently in this animal, the fluid of the central nervous system was removed by drainage from the needle, and the injected oil in the cerebral subdural and subarachnoid spaces abstracted as far as possible. After such removals, the pressure in the subarachnoid space was again determined in the occipital manometer, and oil in different amount was again injected through the small cranial opening. The injections were repeated several times in the animal, with different amounts of the oil, in order to test out the correspondence over a wide range. In all cases, the correction for fluid displaced into the manometer was applied to the

TABLE 3

Table compiled from results obtained on dog (expt. B-1)

This animal, whose cerebrospinal fluid pressure was measured by open-end manometer of 1 mm. bore, was given 30 cc. 30 per cent NaCl intravenously. When the nervous system was dehydrated, bland oil was injected intradurally in various amounts and the pressure-increase in the cerebrospinal fluid determined.

C.S.F. PRESSURE-CHANGE	$\frac{dV}{dP}$	COMPUTED $\frac{dV}{dP} \times$ PRESSURE-CHANGE	CORRECTED COMPUTATION	OIL ACTUALLY INJECTED UNDER DURA
cm.		*cc.*	*cc.*	*cc.*
11.5	0.17	1.79	1.96	1.8
18.3	0.17	3.11	3.41	3.0
15.0	0.17	2.55	2.79	2.6
3.1	0.17	0.52	0.57	0.6
3.9	0.17	0.66	0.72	0.8
12.1	0.17	2.06	2.26	2.2
24.0	0.17	4.08	4.45	4.4

calculations; the correction was relatively small and did not disturb the correspondence between the theoretical and actual determinations. The whole series of results is given in table 3.

In other cases, the exact agreement of results noted in this experiment was not obtained. This can probably be attributed to slight alterations in the elasticity of the system in the course of the experiment and consequent alterations in the value of the fraction $\frac{dV}{dP}$.

This fraction would only remain equal to 0.17 throughout an entire experiment in ideal cases and would vary in value from dog to dog owing to differences in volume V. In the great majority of cases, however, a number of observations gave results of striking identity between the actual amount of fluid injected and the computed amount as determined

by multiplication of the pressure-change by the fraction $\frac{dV}{dP}$ with its average value of 0.17.

In still other experiments, the introduction of fluid into the space about the dehydrated nervous system was made from the manometer and in these cases normal saline solution was substituted for the mineral oil. Here again some inconstancy of results was obtained; but on the whole the determination of the volume-change effected by means of the hypertonic solutions was amazingly accurate as judged by the correspondence between the amounts of foreign solution actually injected with the calculated value as derived by multiplying the observed pressure-increase by the factor 0.17.

Tambour-tube system. By use of the glass-tube system with attached tambours, many series of observations were undertaken, employing manometers of different bores for comparison with the findings as obtained by the bubble-manometer. The elasticity of the system, represented in the covering of the tambour, was a determining factor in the magnitude of the reactions, both in the system which permitted no external dislocation of fluid and in that permitting dislocation of fluid into the open-end manometer. Not only were experiments made with a single tambour attached at either end, corresponding to the tail or head position of the living animal, but other observations with a tambour at each end were carried out. The results were quite similar; and, like the experiments recorded previously (Weed and Flexner, 1932), no essential difference was observed on tiltings from the horizontal to either of the vertical positions.

In such a system of rigid tubes and elastic membrane attached in the tail position, the difference on tilting between the readings with the bubble-manometer and those with the open-end manometer of 1 mm. bore was far greater than in the living animal. As one employed manometers of larger and larger bore, the pressure-changes diminished quite as they did in the animal series. Furthermore, under the conditions of experimentation, the membrane was distended in the horizontal position in order that a positive pressure might be obtained. In those experiments in which almost no dislocation of fluid occurred, this distention of the membrane had no influence on the character of the shift,—that is, on whether the shift was to the vertical head-down or tail-down position. When however manometers of larger bore were employed, with a greater dislocation of fluid, the head-down shifts almost always gave a larger increase in pressure than the decrease in pressure obtained on shifts from the horizontal to the vertical tail-down position. This finding was interpreted as being due to the fact that in such shifts to the tail-down position fluid was dislocated from the manometer into the glass tube-tambour

system causing further distention of the membrane at the caudal end of the system. This further distention taxed the elasticity of the membrane more than the partial collapse of the distended membrane when fluid was dislocated into the manometer in the contrariwise shift. The difference in response of the rubber membrane offers the explanation for this difference in pressure-alterations.

As computations had been made on the constancy of the factor $\frac{dV}{dP}$ in experimental animals on such tiltings from the horizontal to the vertical positions, similar calculations were made from the pressure-alterations derived from the rigid tube-tambour system when connected to the various

TABLE 4

Record of pressure-readings obtained from glass tube-manometer system for determination of $\frac{dV}{dP}$

Glass tube of 400 mm. length; single tambour of 35 mm. diameter attached in "tail" position. Tambour covered with glove rubber of medium thickness.

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN			
	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*	
Bubble	36.5	0	0		36.3	0	0	
1 mm.	28.1	8.4	0.488	0.058	27.8	8.5	0.483	0.057
2 mm.	27.0	9.5	0.572	0.060	26.6	9.7	0.564	0.058
4 mm.	14.4	22.1	1.428	0.064	11.6	24.7	1.151	0.047
6 mm.	7.0	29.5	1.848	0.062	5.8	30.5	1.531	0.050
8 mm.	3.9	32.6	1.985	0.061	3.2	33.1	1.628	0.049
10 mm.	2.8	33.7	1.994	0.059	2.3	34.0	1.637	0.048

types of manometers. The results of a single experiment of this type are given in table 4 for the tiltings from the horizontal to the two vertical positions, with the bubble manometer and with the open-end manometers of various bores.

The remarkable constancy of the factor $\frac{dV}{dP}$ in this physical system, simulating the experimental animal, is of great interest when examined in comparison with the findings given in table 1. For the tiltings from the horizontal to the vertical head-down position, in which some collapse of the partially distended rubber membrane may be assumed, the factor is quite constant. This is true also of the tiltings to the vertical tail-down position with the 1 mm. and 2 mm. manometers. With the larger

manometers, particularly when the dislocation of fluid exceeded 1 cc., the factor becomes slightly smaller, but is still well within the limits of the experimental error.

It was found that when the elasticity of the system was altered by using different rubber membranes or by using tambours of different diameters, factors $\frac{dV}{dP}$ of entirely different magnitude were derived, yet these factors also were remarkably constant for each experiment.

Discussion. The results reported in the foregoing pages seem quite striking in showing a relation between the dislocation of the cerebrospinal fluid and its pressure. One observation of practical importance is the fact that, with the usual manometer of 1 mm. bore, pressure-changes in the cerebrospinal fluid, even those of great magnitude, are quite similar to the readings obtained with the bubble-manometer where no dislocation of fluid occurs. These results are of interest in demonstrating that the use of this open-end manometer is satisfactory for observations of the changes occurring in the cerebrospinal fluid in most types of experimentation.

Aside from these more or less practical considerations, the series of experiments on the living animal demonstrated a definite and fairly constant relationship between the dislocation of cerebrospinal fluid and its pressure, expressed in the ratio $\frac{dV}{dP}$. This ratio, computed from the change in volume of the dislocated fluid, divided by the change in pressure, is theoretically a function of the total volume V and of the elasticity of the system. In any one animal or in those of uniform size, where V does not vary appreciably, the fraction $\frac{dV}{dP}$ should remain constant provided no alteration in elasticity occurs. This was found to hold true. Hence, having calculated the value of this fraction for one normal animal, it should be possible to apply it to other animals of approximately the same size and weight, with systems of normal elasticity, for calculation of the decrease in volume of the intradural contents occurring as a result of dehydration. This was done successfully and demonstrates the interesting and important fact that, within the same species, the elasticity factor is constant enough in normal animals to permit calculations of volume-changes from observed pressure-changes. Consequently, observations made simultaneously on pressure and volume changes should permit a theoretical determination of total volume.

In the course of these experiments it was found that a similar fraction had been calculated by Ayala (1923) in studies of the cerebrospinal fluid in clinical cases. In his first paper he computed what he called a Rachidealquotient, $\frac{Q \cdot F}{I}$, where Q is the amount of cerebrospinal fluid removed,

F the final pressure, and I the initial pressure. Ayala could not assign any definite mathematical value to his quotient, $\frac{Q \cdot F}{I}$; but as it varied directly with the fluid removed and with the final pressure, as well as inversely with the initial pressure, Ayala considered that it had clinical value in giving an idea of the relationship between the quantity of cerebrospinal fluid and its pressure. In a long series of cases the quotient was found by Ayala to range from 1.66 to 20.69, depending upon the character of the pathological lesion within the nervous system.

While the variation in Ayala's Rachidealquotient seems extreme in pathological conditions, the index has some diagnostic importance in the hands of various competent observers (cf. Ayer, 1926; Balduzzi, 1924). Later Ayala (1925) modified the quotient, deriving it by dividing the difference between the initial and final pressure by the quantity of cerebrospinal fluid removed by lumbar puncture. This is the reciprocal of the fraction $\frac{dV}{dP}$ used in this paper. Ayala stated correctly that the value of his quotient is an expression of the volume of the cerebrospinal fluid and the elasticity of the system. Assuming that physiological elasticity does not vary greatly, he considered the quotient as an index of volume and found, from his clinical results, an indication of smaller volumes in cases of brain tumor. As he dealt only with pathological cases the value of his quotient was not constant and the variations he found may have been due to changes in volume, as he supposed, or to changes in both volume and elasticity.

In our experiments, holding the volume as invariable as possible by using animals of the same size, we have demonstrated that the value of the fraction is remarkably constant, indicating but little variation in the elasticity of the physiological system in normal animals of the same species. Once the value of the coefficient of elasticity is determined, the formula can be applied to a calculation of volume-change with change in pressure, or to a calculation of total volume from simultaneous volume and pressure-changes.

The very similar results obtained with a system of rigid tubes and rubber membranes show that the physiological mechanisms involved can be considered as elastic systems. They apparently concern the elasticity not only of the anatomical coverings of the nervous system (the cerebrospinal fluid channels) but also of the blood vascular system. The elasticity of the dura against outward distention seems to be very small indeed. Particularly is this true in the intact animal, for in the cranial region the dura is closely applied to the bony skull and therefore constitutes a membrane of great rigidity—surely of far greater rigidity than the spinal dura, suspended in the epidural space with its areolar tissue and thin walled

veins. The fact that there is in most animals a constant relationship between the fluid dislocated and the resultant pressure of the cerebrospinal fluid indicates a considerable elasticity which permits a certain dislocation of fluid even in the intact animal (Weed, 1929a, b; Weed and Flexner, 1932). This dislocation of fluid may be due to the elasticity (collapse inward) of the spinal dura on the vertical head-down tiltings, but it also seems related fundamentally to the possible compression or dilatation of the blood vessels. Because of the high pressure existing in the arteries of the central nervous system in comparison to that of the veins, it would seem important to emphasize the elasticity of the venous system as a factor in the establishment of the relation of dislocation of the fluid to its pressure rather than the elasticity of the arterial channels.

In those animals in which the fraction $\frac{dV}{dP}$ is constant, it seems safe to assume an elasticity of the system almost as perfect as that of the rubber membranes in the tambours. Were the elasticity of an imperfect character, varying with different loads and with different amounts of dislocation of fluid, no constant fraction could possibly be obtained.

We have then in the central nervous system a mechanism which relates the dislocation of cerebrospinal fluid to the resultant pressure. The constancy of this relationship is remarkable in those animals where the intradural volume is the same: it indicates that the whole central nervous system may be looked upon as enclosed within elastic membranes whose function is modified by the rigid character of the bony encasement.

SUMMARY

Pressures of the cerebrospinal fluid were measured by bubble manometer and by open-end manometers of various bores in dogs of similar weight and size. Pressures recorded in manometers of 1 mm. bore did not vary greatly from those obtained with the bubble manometer. With larger open-end manometers, the pressure-alterations in the cerebrospinal fluid on tilting from the horizontal to the vertical positions, decreased in proportion to the amount of fluid dislocated in the manometer.

In animals of the same size there was a constant relationship between volume of fluid dislocated and pressure-change. By means of this relationship, expressed in the fraction $\frac{dV}{dP}$, accurate determinations were made of the volume-diminution in brain and cerebrospinal fluid, effected by intravenous hypertonic solutions. The value of the fraction $\frac{dV}{dP}$ is a function of the volume of the system and of its physiological elasticity.

The pressure-changes, on tilting from horizontal to vertical positions, were duplicated by an artificial system of glass tubes and tambours

covered with rubber membranes. From this system a similar constant, expressing the relationship between fluid-dislocation and pressure-change was obtained, the magnitude of the fraction depending on the elasticity and capacity of the membranes.

BIBLIOGRAPHY

AYALA, G. 1923. Zeitschr. f. d. ges. Neurol. und Psych., lxxxiv, 42.
1925. Monatsschr. f. Psych. und Neurol., lviii, 65.

AYER, J. B. 1926. The human cerebrospinal fluid. Chap. xi, p. 159. (Assoc. Research Nerv. & Ment. Dis., vol. iv, N. Y.)

BALDUZZI, O. 1924. L'Encéphale, xix, 83.

WEED, L. H. 1929a. Intracranial pressure in health and disease. Chap. iii, p. 25. (Assoc. Research Nerv. & Ment. Dis., vol. viii, Baltimore.)
1929b. Arch. Surg., xviii, 1049.

WEED, L. H. AND P. S. MCKIBBEN. 1919a. This Journal, xlviii, 512.
1919b. This Journal, xlviii, 531.

WEED, L. H. AND W. HUGHSON. 1921a. This Journal, lviii, 53.
1921b. This Journal, lviii, 85.
1921c. This Journal, lviii, 101.

WEED, L. H. AND L. B. FLEXNER. 1932. Bull. Johns Hopkins Hosp., l, 196.

WEIL, A., F. R. ZEISS AND D. A. CLEVELAND. 1931. This Journal, xcviii, 363.

HETEROTOPIC BONE FORMATION IN THYROPARATHYROIDECTOMIZED DOGS

CARL A. DRAGSTEDT AND JOHN E. KEARNS, JR.

From the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication December 18, 1931

We have been attempting by a variety of methods to study the relationship of the parathyroid glands to calcification and ossification. One method that suggested itself as being free from some of the objections to experiments on skeletal growth or repair of fractures in which the questions of osteogenesis from preëxisting bone, periosteum or cartilage may complicate the interpretation, was that of ectopic or heterotopic bone formation. As Phemister (1923) has stated, there exists a tendency to ossification in the organs of the urinary tract, under certain experimental and pathological conditions, which is greater than that in any other system of organs. Two particular types of experimental ossification that have been developed which illustrate this tendency on the part of the urinary tract have been studied. Neuhof (1917) reported the formation of bone in fascial transplants which were used to repair a defect in the wall of the urinary bladder of dogs. This was a consistent finding, occurring in all of eighteen dogs that survived the operative procedure for a long enough period. He reported calcification occurring as early as six days, and ossification occurring as early as seventeen days in some animals, but the height of ossification occurring between six and eight weeks. This has been confirmed by others, notably Phemister (1923), who reported bone formation in every instance even though attempts were made to keep the urine alkaline by diet and administration of alkalis. Huggins (1930, 1931) has confirmed and extended these observations and has shown that bone will form in a fascial implant in the urinary bladder even though the ureters are transplanted so that no urine enters the bladder. In addition Huggins has shown that when an excised portion of the bladder wall or bladder mucosa is stitched so that it overlies the rectus fascia, a cyst will form in the course of twenty days or more, and that in a portion of the wall of this cyst, bone will invariably develop if infection does not occur.

We have performed a number of experiments similar to those of Neuhof and Huggins in two series of thyroparathyroidectomized dogs. A circular patch of the bladder wall about 2 cm. in diameter was excised from the dome of the urinary bladder. This defect was then filled by stitching in a

circular patch of fascia lata with chromic catgut. The excised portion of the bladder wall was then stitched to the anterior sheath of the rectus abdominis muscle. Both procedures were carried out in some animals, while in others only one was performed. For convenience in reporting the results in tabular form we have designated the patch of fascia lata transplanted into the wall of the urinary bladder as a "Neuhof patch," and the patch of the urinary bladder wall stitched to the rectus fascia as a "Huggins patch." In one series of thyroparathyroidectomized dogs intensive calcium therapy was instituted. Calcium lactate was given by mouth, with supplementary calcium gluconate[1] intramuscularly or intravenously. An effort was made to keep the animals free of tetany and to keep the level of the blood calcium as near the normal as possible. The results in this series are indicated in the following table:

TABLE 1

Heterotopic bone formation in thyroparathyroidectomized dogs. Intensive calcium therapy

N = "Neuhof Patch." H = "Huggins Patch."

DOG NUMBER	DAYS	RESULT
1*	63	Bone present—H
2	49	Bone present—N
3	56	Bone present—N and H
4	62	Bone present—N
5	63	Bone present—N
6*	66	Bone present—N and H
7	59	Bone present—H

* Dogs 1 and 6 did not show tetany during test periods of calcium withdrawal. Figure 1 illustrates the bone formation typical of this group.

In the second series of thyroparathyroidectomized dogs, the effort was made to keep the animals alive with minimal calcium administration, with the object of maintaining low blood calcium levels for as much of the time as possible. As is indicated in the following table a number of animals succumbed from tetany before a sufficiently long period elapsed to draw conclusions. It was possible, however, to get several to survive for the period ordinarily required for bone formation.

DISCUSSION. It is not pertinent to this paper to discuss the local factors which condition these types of heterotopic osteogenesis. Readers are referred to Huggins (1931) for a consideration of this aspect of the problem. It is quite clear, however, from the experiments in the first series of dogs that heterotopic bone formation can and does occur in the

[1] We are indebted to Mr. E. D. Marti of the Sandoz Chemical Works for the supply of calcium gluconate used in these experiments.

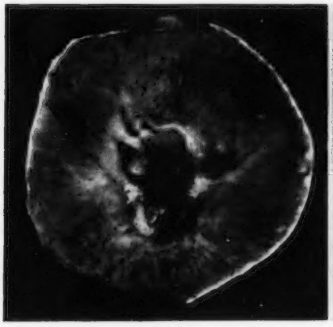


Fig. 1

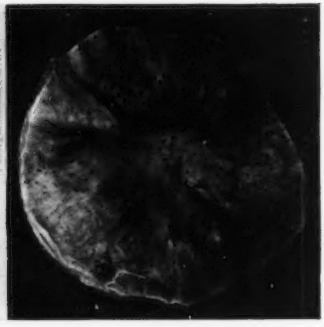


Fig. 2

Fig. 1. Portion of urinary bladder wall of dog showing fascial implant ("Neuhof patch"). Thyroparathyroidectomy with intensive calcium administration and resulting bone formation.

Fig. 2. Portion of urinary bladder wall of dog showing fascial implant ("Neuhof patch"). Thyroparathyroidectomy with minimal calcium administration and no bone formation.

TABLE 2

Heterotopic bone formation in thyroparathyroidectomized dogs. Minimal calcium therapy

N = "Neuhof Patch." H = "Huggins Patch."

DOG NUMBER	DAYS	RESULT
1*	60 and 210	No bone—N
2	19	No bone—N
3	8	No bone—N and H
4	3	No bone—H
5	9	No bone—H
6	6	No bone—N and H
7	25	No bone—N
8	4	No bone—N
9	4	No bone—N
10	5	No bone—N
11	6	No bone—N
12	10	No bone—H
13	5	No bone—H
14	2	No bone—H
15	22	No bone—N
16	2	No bone—N and H
17	64	Bone present—N and H
18	56	No bone—N

* Dog 1. Exploratory laparotomy at 60 days. Sacrificed at 210 days. Figure 2 illustrates a fascial implant into the urinary bladder in which no bone was formed.

dog in the absence of the parathyroid glands if ample calcium is given to maintain the animals free of tetany. Two of the dogs in this group probably did not have a complete parathyroid deficiency as they did not show tetany during a test period when calcium was withheld. The remaining five were apparently completely deficient with respect to parathyroid tissue as demonstrated by repeated attacks of tetany during such test periods. The amount of bone found in these thyroparathyroidectomized dogs seemed in most instances to be practically as great as in similar experiments on normal animals.

From the experiments in the second series there is some indication that this heterotopic bone formation is less likely to occur in thyroparathyroidectomized dogs maintained with minimal calcium therapy. Three animals (nos. 1, 17, and 18) survived a long enough period for bone formation to regularly occur in normal animals, but bone developed in only one. Three additional animals (nos. 2, 7 and 15) survived a long enough period for bone formation to occur frequently in normal animals, but no bone developed. With respect to one of the dogs in this series (no. 1) that did not develop bone in a "Neuhof patch," it can be definitely stated that the blood calcium level was below normal at all times. In this instance the thyroparathyroidectomy had been performed seven months before the operation on the urinary bladder. Calcium administration had been discontinued for a month and was not required to prevent tetany during any of the time that the experiment continued. The blood calcium levels varied between 5.1 and 7.2 mgm. per 100 cc. except on one occasion when a value of 9.1 was observed. In the other animals subnormal blood calcium levels probably occurred during most of the experimental period as tetany was frequent and the minimal amount of calcium administered that would alleviate it.

CONCLUSIONS

1. Heterotopic bone formation can occur in thyroparathyroidectomized dogs receiving adequate calcium therapy in *a*, fascial transplants in the wall of the urinary bladder, and in *b*, the cysts formed by stitching the urinary bladder mucosa to the rectus fascia.

2. There is definite indication that such heterotopic bone formation is less likely to occur in thyroparathyroidectomized dogs receiving minimal calcium therapy.

BIBLIOGRAPHY

HUGGINS, C. B. 1930. Proc. Soc. Exper. Biol. and Med., xxvii, 349.
1931. Arch. Surg., xxii, 377.
NEUHOF, H. 1917. Surg., Gynec. and Obst., xxiv, 363.
PHEMISTER, D. B. 1923. Annals Surg., lxxviii, 239.

THE SPECIFICITY OF PANCREATIC LIPASE: ITS APPEARANCE IN THE BLOOD AFTER PANCREATIC INJURY

IAN S. CHERRY AND LATHAN A. CRANDALL, JR.

From the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication January 9, 1932

Since the work of Hanriot (1897), Doyon and Morel (1902), and Arthus (1902) it has been known that the serum contains normally no enzyme capable of hydrolyzing the true fats or oils, differing in this respect from pancreatic secretion which is very active on the triglycerides of the higher fatty acids. These same workers recognized that an esterase capable of splitting ethyl butyrate was present in the blood. At the present time, however, it is a common practice to use ethyl butyrate, tributyrin, or triglycerides of the higher fatty acids indifferently as substrates for an enzyme which is referred to as lipase. Enzymes are chiefly distinguished by the substrates on which they act, and Oppenheimer (1925) states that since serum esterase is active on such triglycerides of the lower fatty acids as tributyrin the difference between this esterase and the lipase which hydrolyzes true fats may be questioned.

If the lipase of the pancreas is a specific enzyme and essentially different from the serum esterase one might expect that a lipase attacking true fats would appear in the blood following pancreatic injury and that the concentration of such a lipase in the serum would not parallel that of the normal serum esterase. An increase in serum diastase is known to occur following pancreatic injury; the literature has been reviewed recently by Elman, Arneson, and Graham (1929). Several workers (Hess, 1912; Hiruma, 1923; Diena, 1919) have attempted to demonstrate an increase in blood lipase after pancreatic duct ligation. All of them used the simple esters as substrates, and their results are not in agreement for reasons which will appear. Hewlett (1904) found an increase in esterase (ethyl butyrate) in the urine after pancreatic injury and in one case where olive oil was used as a substrate a lipase was demonstrated.

With these facts in mind it was considered of considerable importance to determine whether "pancreatic" lipase (olive oil splitting lipase) appears in the blood after pancreatic injury.

METHOD. The outflow of secretion from all or part of the pancreas was obstructed in 10 dogs. In 6 of these both pancreatic ducts were ligated, in

one animal the tail of the pancreas was doubly ligated and cut between ligatures, and in the remaining three the head and tail were both ligated and the body of the pancreas removed, leaving head and tail free in the mesentery. Determinations of the lipolytic activity of the serum were made prior to operation and at frequent intervals postoperatively, using ethyl butyrate and olive oil as substrates. As further controls more than 50 determinations on 30 normal animals under similar conditions of diet and subjected to various surgical procedures (gastrectomy, biliary fistula, section of splanchnics, ureteral ligation, London cannula on portal vein) were made.

A modification of the Loevenhart method for esterase was employed; to 1 cc. of serum were added 3 cc. distilled water, 1 cc. absolute ethyl butyrate, and 0.5 cc. N/3 potassium phosphate buffer adjusted to pH 7.0 (3 cc. N/3 potassium dihydrogen phosphate plus 10 cc. N/3 potassium hydrogen phosphate). This mixture was shaken thoroughly and allowed to stand in the incubator at 40°C. for 24 hours. It was then titrated with N/20 sodium hydroxide, using 3 drops 1 per cent phenolphthalein as an indicator and bringing the solution to the faintest permanent pink.

Some difficulty was experienced in finding a preparation of olive oil which would undergo no change on standing, which would be a sufficiently fine emulsion to present the maximum surface to the action of the lipase and would not settle out to any marked extent on standing, and which could be readily duplicated when more material was required. A 50 per cent emulsion of olive oil in water using 5 per cent of acacia as the emulsifying agent was found to be suitable. The olive oil was freed from all but faint traces of free fatty acid before use. A preservative was necessary and it was found that sodium benzoate was highly satisfactory, not inhibiting the lipolytic action nor interfering with the stability of the emulsion; the concentration of sodium benzoate used was 0.2 per cent. The emulsion was run through an homogenizer 10 to 12 times, which resulted in a very fine and uniform size of oil droplets. The emulsion as described was kindly prepared for us by Dr. D. L. Tabern of the Abbot Laboratories. In testing the action of lipases on olive oil 2 cc. of the above emulsion were added to 1 cc. of serum, and 3 cc. of distilled water and 0.5 cc. of normal phosphate buffer pH 7.0 added as in the ethyl butyrate method. Incubation and titration were the same as for the ethyl butyrate method except that 3.0 cc. of 95 per cent alcohol were added to each tube before titrating.

A series of substrates was employed to determine whether further information on the specificity of the lipases or esterases of serum, liver, and pancreas could be obtained. In all cases the method described for ethyl butyrate was used, substituting 1 cc. of the ester to be studied for the 1 cc. of absolute ethyl butyrate. Extracts of liver and pancreas were made as described below. The esters studied were ethyl, amyl and benzyl

acetate, methyl and iso-amyl salicylate, tributyrin, triacetin, iso-amyl caproate, benzyl succinate, benzyl butyrate, ethylene glycol diacetate, iso-amyl benzoate, ethyl mandelate, ethyl butyl and octyl esters of olive oil, benzyl oleate, and ethyl and butyl stearate.

Since Rona and his co-workers believe that tributyrin is an adequate substrate for pancreatic lipase a separate series of experiments was designed to test this assumption. Determinations of serum lipolytic activity were made in 7 dogs, 3 with ligation of both pancreatic ducts and 4 with ligation of the common bile duct.[1] The hydrolysis of tributyrin was followed both by the titration method as above and by stalagmometry; parallel observations on the hydrolysis of ethyl butyrate and olive oil were carried out.

Other determinations were made to study the distribution of olive oil and ethyl butyrate splitting enzymes in various tissues. Brain, lung, muscle, kidney, spleen, liver, intestinal mucosa, and pancreas were utilized. To 20 grams of the ground tissue 80 cc. of glycerol were added, the mixture shaken and allowed to stand 24 hours. For use the extract was diluted with an equal volume of water and 1 cc. used in place of serum in the methods described above. A similar study, much more extensive in scope, has been reported by Porter (1916). Unfortunately she did not adjust the pH by the use of buffer, and the adequacy of her reported control determinations may be questioned. She found enzymes capable of splitting true oils and fats to be present in varying amounts in various animal species, such a lipase being present in at least one species for each tissue examined. The highest values were obtained from pancreas, liver, and thymus. She did not study dog tissues.

In all the above experiments control tubes employing serum or tissue extract inactivated by heat were used. After a large number of controls had been run on serum, it was found that control tubes employing the same lot of substrate were constant within 0.1 cc. N/20 NaOH; since this was within the limits of error of the determinations, one control was allowed to serve for a number of sera.

Results. Data on 5 of the dogs with pancreatic damage are presented in table 1; results on the other 5 (all with simple duct ligation) are similar. The important fact demonstrated is that in all these animals an olive oil splitting lipase appeared in the blood, the amounts found being surprisingly large. It is interesting to note that in 3 of the 5 animals the maximum value was reached within 24 hours after the operation. Dog 3 in which the maximum value was not reached until the 5th day, refused food postoperatively and died on the 7th day of pneumonia and fat necrosis. Gould and Carlson (1911) following diastase after obstruction to the outflow of

[1] We have found that hepatic injury causes an olive oil splitting lipase to appear in the serum. These observations will subsequently be reported in detail.

pancreatic secretion noted that a secondary rise frequently occurred after the diastase had fallen to normal; the same phenomenon occurred in dog 4 on the 13th day, the lipase rising abruptly almost to its first high level after a gradual postoperative fall. The ethyl butyrate esterase may fall concomitantly with the rise of olive oil lipase, may rise simultaneously, or may show almost no change. Esterase changes, however, are always relatively slight. In our series of 30 control animals, 2 have shown spon-

TABLE 1

Blood esterases in dogs following pancreatic injury

Results expressed in cc. N/20 NaOH. Values for olive oil lipase in columns OO, for ethyl butyrate lipase in columns EB.

DAYS	DUCTS LIGATED		TAIL LIGATED		BODY OF PANCREAS EXCISED					
	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5	
	OO	EB	OO	EB	OO	EB	OO	EB	OO	EB
Control	0.0	2.1	0.0	1.4	0.0	2.3	0.0	1.8	0.0	2.0
1	6.1	1.7	1.0	1.8	0.6	2.0	7.4	1.8	5.0	1.9
2	6.0	0.9	1.2	1.3	0.9	1.8	5.0	1.7		
3	6.6	1.0	0.6	1.6	1.2	1.8	1.8	1.6	5.0	1.7
4			0.2	1.5	1.6	1.8	1.3	1.7		
5	2.0	1.4	0.4	1.3	4.8		1.1	1.7		
6	1.7	2.1	0.1	1.5	1.9	1.4	1.7	1.5		
7	1.1	2.4			Died		1.4			
8	0.4	1.8							2.3	1.5
9	0.0	3.7					2.9	1.8		
10	0.3	3.8								
12	0.1	2.0					0.8	2.1	0.5	2.3
13							6.0	1.9		
14	0.1	2.7								
15							5.6	3.0		
17	0.0	1.2					5.3	6.3	0.4	
20	0.0	1.8					3.8	3.4		
25							3.4	1.8		
29							1.8	3.2		
34							1.8	3.2		
39							3.0	3.7		
42							0.9			

taneously a slight amount of "pancreatic" lipase in the blood; in more than 50 determinations on 28 other animals, including many subjected to various surgical procedures, we have never observed any olive oil lipase in the blood except after liver injury

Our study of 21 various esters showed that in general liver tissue is more active on the simpler esters than are pancreas or serum; the esters of olive oil, oleic acid, and stearic acid were split only very slightly by any of the three; while olive oil emulsion is acted on very strongly by pancreas,

moderately by liver, and not at all by serum. The salicylic acid esters were split only by liver, which Loevenhart (1906) has shown is due to the presence of bile salts. It must be borne in mind that the menstruum in which the enzyme acts may exert a very profound modifying influence in an experiment such as this.

Data on 2 of the seven dogs in which parallel determinations of activity on olive oil, ethyl butyrate, and tributyrin (titration and stalagmometric methods) were made are given in table 2; these results are typical of the group. It is our impression that the stalagmometric method is no more accurate than titration for ethyl butyrate determinations and it is certainly much more laborious.

TABLE 2

Comparative determinations of the activity of serum before and after ligation of the pancreatic ducts on olive oil, ethyl butyrate, and tributyrin (titration and stalagmometric methods)

Results of titration on olive oil, ethyl butyrate, and tributyrin given as cc. N/20 NaOH in 24 hours; on tributyrin by stalagmometry as drops in 1 hour.

DAYS	DOG P19				DOG P20			
	Olive oil	Ethyl butyrate	Tributyrin		Olive oil	Ethyl butyrate	Tributyrin	
			Titration method	Stalagmometric method			Titration method	Stalagmometric method
Control 1	0.1	1.3	2.0	13	0.0	2.0	3.0	18
Control 2	0.0	1.5	2.4	14	0.0	1.8	3.3	21
Postop. 1	3.4	1.3	2.6	15	4.4	2.1	3.6	24
2	4.4	2.0	3.5	18	5.1	2.0	3.7	22
3	4.6	2.2	3.7	22	5.2	1.8	3.6	23
4	1.8	1.6	2.8	19	5.6	2.1	3.4	24
12					0.0	4.4	5.0	29
14					0.1	3.4	4.5	26
16					0.1	4.3	4.8	28

DISCUSSION. We believe that the appearance of an olive oil splitting lipase in the blood after pancreatic obstruction without a definite parallel change in the serum esterase is the best evidence thus far presented for the specificity of the former enzyme. The fact that we have observed increase, decrease, or no change in the esterase under these conditions explains the discordant results of previous investigators who have used simple esters for the study of pancreatic lipase in the blood. Willstatter and Memmen (1924) and more recently Gyotoku (1930) have shown that the action of quinine and atoxyl on the lipolytic enzymes is much modified by accompanying impurities; this fact must be carefully considered in a comparative study of different sources of enzyme. Since under the ex-

perimental conditions which we have used pancreatic lipase appears in and then disappears from the same medium (serum) in which esterase is present without definite alteration of esterase activity, further weight is added to the evidence for specificity. Conditions are certainly more comparable when enzyme concentrations are independently varied in such a relatively constant medium as blood serum than when the sources of the enzymes are extracts of various tissues.

In view of the distinction which may be made between the two types of lipolytic activity, it seems advisable to reserve the term "lipase" for the enzyme active on triglycerides of the higher fatty acids and "esterase" for the enzyme or enzymes which hydrolyze the simpler esters. Hydrolysis of olive oil is accomplished in significant amounts only by pancreas, intestinal mucosa, liver, and spleen, with a trace of activity in the kidney. All tissues studied showed activity toward ethyl butyrate. We are now

TABLE 3

Distribution of olive oil splitting lipase and ethyl butyrate splitting lipase in the tissues

TISSUE	OLIVE OIL	ETHYL BUTYRATE
	cc. N/20 NaOH	*cc. N/20 NaOH*
Pancreas	10.0	1.4
Pancreatic juice	11.5	3.0
Liver	2.4	14.6
Serum	0.0	2.4
Kidney	0.4	6.0
Spleen	2.1	1.7
Lung	0.2	4.1
Intestinal mucosa	6.0	4.0
Brain	0.2	0.3
Striated muscle	0.1	0.8

attempting to determine whether the pancreas is the sole site of lipase formation.

The possibility that several enzymes are concerned in the hydrolysis of the simple esters is suggested by such observations as those of Falk (1924) who found that various tissue extracts hydrolyze different esters in varying amounts. Our results, however, show no significant variation between the hydrolysis of ethyl butyrate and tributyrin by serum following pancreatic obstruction. The parallel rates of hydrolysis of these two esters, on the contrary, suggest that a single enzyme is responsible. Rona and Pavlovic (1922, 1924) using the stalagmometric method with tributyrin as the substrate find that "serum lipase" is inactivated by both quinine and atoxyl, while atoxyl inactivates "liver lipase" and quinine inactivates "pancreatic lipase." These findings have been applied to the diagnosis of diseases of the liver and pancreas on the theory that the specific

lipases appear in the blood, but with highly conflicting results (Segenschmid, 1927; Lowenberg and Kwilecki, 1926; Kwilecki, 1926; Kobryner, 1927; and Grassberger, 1928). The so-called "pancreatic lipase" has been found in serum in pernicious anemia by Simon (1925) and in secondary anemia by Lasch (1928). In a careful experimental study Kromeke (1923) was unable to find any change in the quinine fast lipase following various types of liver injury. The unpredictable behavior of esterase under various conditions may explain the conflicting results of those who have used the stalagomometric method. It is evident from our data that tributyrin hydrolysis is not an index of the presence of true pancreatic lipase. In our hands the method which depends on changes in surface tension (stalagmometry) has no superiority either in accuracy or ease of determination over titration. The division into atoxyl resistant and quinine resistant fractions is based on the properties of organ extracts, and may easily be due to differences in the menstruum.

It is possible that the appearance of lipase in the blood after pancreatic obstruction may be due to the presence under those conditions of an activating agent; we think it unlikely that this is actually the case.

CONCLUSIONS

1. Following obstruction of the pancreas in dogs an olive oil splitting lipase appeared in the blood in large amounts; there was no significant uniform change in esterase as measured by hydrolysis of ethyl butyrate or tributyrin.

2. This is interpreted as evidence of the specificity of lipase; it is suggested that this term "lipase" be reserved for the enzyme (or enzymes) capable of splitting true fats and oils, and that the term esterase be used for the enzyme (or enzymes) acting upon the simple esters.

3. Lipase (olive oil splitting) was found in significant amounts only in pancreas, intestinal mucosa, liver, and spleen. All tissues studied contained an esterase.

4. A study of the activity of pancreatic and liver extracts and blood serum on 21 different esters gave no evidence of specificity other than that indicated by the absence of activity toward olive oil in certain tissues and its presence in others.

BIBLIOGRAPHY

ARTHUS, M. 1902. Compt. Rend. Soc. Biol., liv, 381.
DIENA, G. 1919. Zeitschr. Biochem. u. Biophys., xx, 292.
DOYON, M. AND A. MOREL. 1902. Compt. Rend. Soc. Biol., liv, 498.
ELMAN, R., N. ARNESON AND E. A. GRAHAM. 1929. Arch. Surg., xix, 943.
FALK, K. G., H. M. NOYES AND K. SUGIURA. 1924. Journ. Biol. Chem., lix, 943.
GOULD, L. K. AND A. J. CARLSON. 1911–1912. This Journal, xxix, 165.
GRASSBERGER, A. 1928. Mitteil. a. d. grenzgeb. Med. u. Chir., xli, 1.

GYOTOKU, K. 1930. Biochem. Zeitschr., ccxvii, 279.
HANRIOT, M.-F. H. 1897. Compt. Rend. Acad. de Sci., cxxiv, 778.
HESS, C. L. 1912. Journ. Biol. Chem., x, 381.
HEWLETT, A. W. 1904. Journ. Med. Res., xi, 377.
HIRUMA, K. B. 1923. Biochem. Zeitschr., cxxxix, 336.
KOBRYNER, A. 1927. Deutsch. Arch. f. klin. Med., clv, 353.
KROMEKE, F. 1923. Arch. f. exp. Path. u. Pharm., c, 77.
KWILECKI, D. 1926. Arch. f. Verdauungskrank., xxxvii, 337.
LASCH, C. H. 1928. Arch. f. klin. Chir., cl, 272.
LOEVENHART, A. S. 1907. Journ. Biol. Chem., ii, 391.
LOWENBERG, W. AND D. KWILECKI. 1926. Med. Klin., xxii, 279.
OPPENHEIMER, C. 1925. Die Fermente und ihre Wirkungen. Leipzig, 5th ed.
PORTER, A. E. 1916. Biochem. Journ., x, 523.
RONA, P. AND R. PAVLOVIC. 1922. Biochem. Zeitschr., cxxx, 225.
1924. Ibid., cxxxiv, 108.
SEGENSCHMID, D. 1927. Med. Klin., vii, 400.
SIMON, H. 1925. Klin. Wochenschr., iv, 2295.
WILLSTATTER, R. AND F. MEMMEN. 1924. Zeitschr. f. physiol. Chem., cxxxviii, 216.

METABOLISM IN YUCATAN: A STUDY OF THE MAYA INDIAN

MORRIS STEGGERDA AND FRANCIS G. BENEDICT

From the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, Long Island, New York, and the Nutrition Laboratory, Boston, Massachusetts

Received for publication January 7, 1932

In the Nutrition Laboratory's comprehensive program for studying the metabolism of various races, one of the most salient observations has been that of the relatively high basal metabolism of male Maya in Yucatan. The earliest investigator to discover this high metabolism was Williams (1), who found that the basal metabolism of 32 male Maya was, on the average, 5.2 per cent above the standards for white men of the same age, weight, and height, living in the northern part of the United States. The other outstanding feature of Williams' study was that he noted in several instances strikingly low pulse rates. Three years after Williams made his observations, the Harvard Expedition in charge of Shattuck (2) took up the study again, with the findings of Williams in mind, and the measurements were concentrated upon those individuals whom Williams had observed to have a pronouncedly high metabolism and a low pulse rate. Although the second study was carried out only with considerable difficulty and with a yield of protocols by no means satisfactory to any of the workers, an analysis of the returns showed that, in general, the findings of the first investigation were completely confirmed, that is, that the metabolism of male Maya on the average is 5 or 6 per cent above the standards for American white men of European descent and that there are occasional evidences of a pronouncedly low pulse rate. It was pointed out, however, that in those few instances where measurements were made on more than one day, there was a tendency for the metabolism to be somewhat lower after the first day. It was felt that possibly we had to deal with a group of individuals who, although appearing outwardly calm and completely relaxed, were considerably disturbed by the experimental technique, simple though it was, and it was thought that as the subject became more familiar with the technique his metabolism might tend to approach more nearly the true basal level.

In the winter of 1931[1] the problem was again attacked with the idea 1, of making further metabolism measurements, if possible, on those men who

[1] At that time the Department of Genetics of the Carnegie Institution of Washington began an anthropometric study of the Maya Indian.

had on the previous occasions shown a high metabolism; 2, of noting any low pulse rates, and 3, most important of all, of studying the problem raised by Shattuck and Benedict as to whether observations continued on a second and a third day would show a tendency for a lower metabolism. Anthropometric measurements were also made on the subjects whose metabolism was measured.

In the present study all the field observations were made by Steggerda, who was well acquainted with the difficulties that might be encountered. The place where the observations were carried out was the same (Chichen Itzá) as in the two earlier studies. Our measurements were made in February, March, and April, those by Shattuck in January, February, and March, and those by Williams in March, April, and July. Thanks to the close coöperation of Marty Dzib, a native assistant, sympathetic accord was established with the various subjects. The success of these measurements was also due in no small measure to the most helpful cooperation of Mrs. Morris Steggerda. Precautions were taken to guard against any surreptitious eating by the subjects, and we are certain that we were dealing with the post-absorptive metabolism in all cases. The apparatus with which the metabolism measurements were made was the same as that used in the former studies, namely, the field respiration apparatus (3). The instrument was thoroughly tested before its use, and our technique was reviewed at the Nutrition Laboratory in the fall of 1930, before the start of the expedition, in order to insure perfect manipulation of the apparatus. Since the importance was recognized of securing the full coöperation of the subject, particularly as regards physical and mental repose, every concession was made to insure his tranquillity. As a result, it was possible to study the basal metabolism of 30 male Maya, and in every instance three periods were obtained on each of three consecutive days.

Physical characteristics of the subjects. The anthropometric measurements that pertain to the interpretation of basal metabolism observations, namely, the measurements of body weight, height, and sitting height, are given in abstract in the first part of table 1. To note whether our subjects were especially over or under weight, the Pirquet index of state of nutrition (4), that is, the pelidisi $\left(\frac{\sqrt[3]{10 \times \text{weight (gm.)}}}{\text{sitting height (cm.)}}\right)$ has likewise been computed and recorded in the table. The general configuration of male Maya has been considered *in extenso* by Williams and Benedict (1). The physical characteristics of the groups of Maya studied on the first, second, and third expeditions, respectively, were on the average as follows:

Age, 24, 22, and 27 years; body weight (without clothes) 53.7, 52.7, and 54.4 kgm.; height 158, 159, and 156 cm.; sitting height (measured) 82.3, 83.5, and 83.5 cm.; pelidisi 99, 97, and 98.

This comparison indicates that the physical characteristics of the three groups of men were much the same and that the Maya as a whole are short

TABLE 1

Basal metabolism of male Maya in Yucatan (1931 expedition)*

SUBJECT	AGE	WEIGHT (WITHOUT CLOTHES)	HEIGHT	SITTING HEIGHT	PELIDISI	PULSE RATE			O_2 PER MINUTE			HEAT PRODUCED PER SQ. M. PER 24 HRS.	DEVIATION (AVERAGE) FROM HARRIS-BENEDICT PREDICTION
						1st day	2d day	3d day	1st day	2d day	3d day		
	years	*kgm.*	*cm.*	*cm.*					*cc.*	*cc.*	*cc.*	*cal.*	*per cent*
M. Dzib**	21	48.1	155	81.3	97	53	54	53	196	191	196	938	−0.9
F. Echemaria	18	43.3	148	78.4	97	53	53	53	173	175	180	917	−4.5
E. May	26	57.7	159	83.5	99	57	53	56	207	222	209	929	−0.2
T. Canul	16	54.8	155	80.1	102	59	57	56	233	229	228	1,047	+7.5
V. Dzib	36	53.1	156	85.9	94	54	54	52	219	216	224	1,011	+14.4
R. Mis	22	52.7	153	81.7	98	66	60	59	209	231	213	1,015	+7.4
E. Puc	27	51.8	149	77.7	103	49	48	49	223	203	210	1,015	+9.7
S. Ceme	21	57.7	157	85.3	98	53	50	60	241	236	251	1,067	+12.1
J. Ceme	35	56.6	158	86.5	95	58	57	56	220	217	219	970	+8.7
D. Balam	24	54.6	153	82.0	99	55	52	60	233	219	245	1,069	+13.6
F. May	27	52.4	161	84.2	96	63	56	53	206	206	205	932	+1.4
M. Canul	24	58.6	158	85.1	98	53	50	54	216	217	230	960	+2.3
G. Chan	28	45.9	146	81.2	95	46	43	46	190	183	181	944	+3.5
T. Ek	27	57.2	160	84.4	99	39	35	34	212	216	210	929	+0.4
J. Cene	25	48.1	151	81.6	95	52	46	51	188	206	199	967	+4.4
G. Ek	37	56.3	163	88.5	93	48	41	41	235	238	226	1,012	+15.1
G. Chi	24	57.8	154	81.5	101	51	46	49	234	219	212	987	+4.8
C. Mucul	25	53.9	149	83.3	98	59	60	54	263	254	237	1,188	+26.1
R. Dzib	40	62.2	165	86.5	98	53	48	47	256	230	232	990	+12.6
M. Dzib	19	64.0	167	88.0	98	65	55	52	256	263	259	1,048	+8.9
B. Tun	31	52.2	155	82.1	98	50	47	48	238	220	217	1,049	+15.8
S. Mex	22	59.5	162	86.8	96	52	50	51	220	205	216	911	−4.0
A. Castro	30	51.8	149	80.1	100	48	46	43	208	221	221	1,038	+13.9
F. Ceme	28	52.2	156	83.0	97	48	50	51	215	209	204	970	+5.8
I. Mex	28	52.2	158	86.0	94	55	53	54	215	211	212	978	+6.7
L. Mex	39	62.6	164	89.2	96	80	73	80	249	251	259	1,046	+18.5
J. Puc	22	54.0	150	81.2	100	60	58	54	228	219	231	1,061	+11.3
D. Dzib	31	51.8	153	84.3	95	47	47	45	217	224	226	1,044	+15.7
A. Dzib	27	54.9	156	82.9	99	55	50	47	243	228	224	1,045	+13.4
F. Tun	36	53.1	155	83.0	98	46	47	47	203	197	212	945	+6.6
Average	27	54.4	156	83.5	98	54	51	52	222	219	220	1,001	+8.4

* Average room temperature during experiments, 21.0°C.; average outdoor temperature, 20.4°C.; the mouth temperatures showed, if anything, somewhat subnormal values.

** M. Dzib also measured on a fourth and fifth day; pulse rate 50 and 54; O_2, 173 and 178 cc.; deviations from prediction, −12.5 and −10.3 per cent.

and stocky. Although it may be argued that Pirquet's standards may not be directly applicable to the Maya, it is evident that there is no obvious disproportion in the body configuration of these men and that, as a whole, they may be classed as well-nourished individuals, not under or over weight. It is particularly important to bear this fact in mind in our subsequent consideration of the metabolism measurements themselves.

The stem length as an index of normality of body form. According to Pirquet (4) the sitting height of an adult white person may be calculated by dividing the measured standing height in centimeters by 2 and adding 5 cm. Obviously with those individuals having a long stem length this standard correction of 5 cm. will be too small. Hence if the difference between the actually measured sitting height and half the standing height is calculated, one can get some hint as to the normality in the general configuration of the individual. For our group of 30 Maya this difference has been calculated and found to range from 2.6 cm. with T. Canul to 8.8 cm. with C. Mucul. The average difference is 5.6 cm. It is safe to assume, therefore, that the stem lengths of these Maya are not abnormally long and that their body configuration is not greatly different from the general configuration characteristic of a group of Whites.

Pulse rate. The average pulse rates, based upon the several observations on each of the three days of experimentation with each subject, are also given in table 1. The average rate of this group of 30 men was 54 beats per minute on the first day, 51 on the second, and 52 on the third. Judging from this evidence alone, one could assume that there was no great difficulty in establishing a normal state of mental and muscular repose with these subjects, even with the novelty of the experimental procedure. There are notable instances where the pulse rate is 45 or below. Thus G. Chan has an average rate for the three days of 45, T. Ek of 36, and G. Ek of 43. A. Castro has a rate on one day of 43 and D. Dzib on one day of 45. The most striking case is that of T. Ek, whose pulse rate on the three successive days was 39, 35, and 34 beats per minute, respectively, values measurably lower even than the low rates noted for this same man by both Williams and Shattuck. In the Williams and the Shattuck series, although there are no pulse rates below 40, there are a number between 40 and 45. It is an established fact, therefore, that these adult Maya not infrequently have a very low pulse rate. If the grand average is calculated of all the records of pulse rate noted with the Maya on this third expedition, these Indians may be considered to have a pulse rate of 52 beats per minute. This grand average represents a large number of counts on three days in the case of each man. The more limited data obtained in Williams' series show an average rate of 55 beats, and those observations reported by Shattuck an average of 56 beats. Of the 136 white men whose basal metabolism measurements have been listed by Harris and Benedict (5),

the pulse rate was recorded for 121 individuals and found to average 61 beats per minute. It would seem, therefore, that the Maya as a whole have a low pulse rate. It is regretted that studies of the heart rate with electrocardiograms could not have been made, particularly in the case of T. Ek. For normal Whites, without heart block, no records of such low pulse rates exist except in those instances noted by Miles (6) with men who had been living on a sub-maintenance diet. Pulse rates as low as 35 were frequently found during the period of undernutrition, and one man had a rate of somewhat under 30. This phase of Maya physiology is worthy of further study, but obviously such study can not be carried out to advantage in the field.

Oxygen consumption. The oxygen values reported in table 1 are averages of three well-agreeing periods on each of three consecutive days. With some of the subjects the average for the first day is higher than the averages for the second and the third days. This is not uniformly the case with all the subjects. In general the oxygen consumption averaged 222, 219, and 220 cc. on the first, second, and third days, respectively. There is no great difference in these averages, and one could not conclude from these results that there is a tendency for the metabolism to become lower in the successive measurements, as was thought possible by Shattuck and Benedict.

Heat production per square meter of body surface. The heat production has been calculated from the average oxygen consumption noted on the three days and, solely because of custom, has been expressed per square meter of body surface per 24 hours. In this calculation the Du Bois height-weight chart (7) has been employed for estimating the body surface, on the assumption that this chart applies with a sufficient degree of accuracy to the Maya type of configuration. The average heat production of the 30 Maya is 1001 calories per square meter of body surface. The values for the different subjects are strikingly uniform. It is doubtful whether greater uniformity would be found with any group of 30 individuals, whether students, men off the street, or men from any group of workers. Thus, the minimum heat production is 911 calories with S. Mex, and the maximum is 1188 calories with C. Mucul. If C. Mucul is omitted, the maximum is 1069 calories.

Deviation of measured from predicted metabolism. The total heat production of these 30 men has been compared with the Harris-Benedict predictions (5) for white men of the same age, weight, and height, and the percentage deviations of the actually measured metabolism from the predicted metabolism are recorded in the last column of table 1. The majority of the percentages are plus. There are only four minus values and, of these, two are about -4.0 per cent and the other two are less than -1 per cent. Of those subjects having a higher metabolism than the prediction, C.

Mucul shows the greatest deviation, +26.1 per cent, and eleven of the other 29 men give values of over +10 per cent. The average for all 30 men is +8.4 per cent. This particular group of Maya, therefore, had a metabolism definitely higher than the North American standards, even higher than that noted in the two earlier Yucatan studies, for Williams reported an average deviation of +5.2 per cent and Shattuck of +5.8 per cent. This distinctly high metabolism was found not on one day but on three consecutive days.

The heat production of the 30 Maya studied on this third expedition was likewise predicted from the Aub and Du Bois standards (8), although the calculations have not been incorportated in table 1. The average percentage deviation of the actually measured metabolism of this group of Maya from the metabolism as predicted by the Aub and Du Bois standards is +5.1 per cent, as compared with the +8.4 per cent when the prediction is made by the Harris-Benedict standards. In both cases the metabolism is definitely plus.

Our subject M. Dzib had been in the United States for 2½ years just previous to these metabolism tests. F. Echemaria, during the time of these observations, was fed at the mess of the Carnegie Institution of Washington and hence was not partaking of native food. These two men show essentially the lowest metabolism in the entire series, but we would not definitely ascribe their low metabolism to either of the above-mentioned facts. This information is recorded, however, as of possible later use in any subsequent studies of the effects of climate and food.

In this third expedition it was possible to study 12 of the same men measured by Shattuck. Seven of these men had likewise served as subjects for Williams. The percentage deviations (from the Harris-Benedict standards) found with these men by the three different investigators are recorded in table 2. The average deviation for the 12 men was found by us to be +7.2 per cent and by Shattuck to be +3.3 per cent. For the 7 men measured in all three expeditions, the deviations averaged +5.7 per cent in the Williams series, +1.5 per cent in the Shattuck series, and +8.1 per cent in our series. In comparing the metabolism as measured by us with that measured by either Shattuck or Williams, one should bear in mind that in our series the averages are in every case the result of three days' observations comprising nine periods in all, whereas in both of the earlier series the values represent frequently only one day or two days and oftentimes only two periods on any given day. We are convinced that some of the birth dates furnished to Shattuck were erroneous and that a number of his subjects were older than recorded in the Shattuck series. This applies likewise to a certain extent to the Williams series. The metabolism predicted by the Harris-Benedict standard for a man of 21 years, for example, will be higher than that predicted for the same man with

an age of 25 years. If his measured metabolism is above the predicted metabolism for the age 21 years, it will therefore be even higher above the prediction for 25 years. This probably explains in part the differences in the average percentage deviations noted for the same subjects in the three expeditions. When it is considered that these three series of measurements were made at intervals of from one to three years, it is surprising that the agreement on the whole is so uniform.

TABLE 2

Comparison of metabolism data secured on the same male Maya by three different investigators

SUBJECT	DEVIATION OF MEASURED FROM PREDICTED (HARRIS-BENEDICT) METABOLISM		
	Steggerda	Shattuck	Williams**
	per cent	*per cent*	*per cent*
E. May*	−0.2	−2.2	±0.0
T. Canul	+7.5	+7.5	
E. Puc	+9.7	+1.7	+6.1
S. Ceme	+12.1	+11.5	+3.3
J. Ceme	+8.7	+6.4	
D. Balam*	+13.6	+11.8	
J. Cene	+4.4	+6.6	
G. Chi	+4.8	+1.1	+11.5
C. Mucul	+26.1	+8.7	+13.7
G. Chan	+3.5	−8.5	+5.1
T. Ek	+0.4	−1.8	−0.1
S. Mex	−4.0	−3.4	
Average	+7.2	+3.3	
Average for same 7 men studied by Williams	+8.1	+1.5	+5.7

* Erroneously recorded as U. May and D. Balm in the report by Shattuck and Benedict (2).

** In the report by Williams his subjects were not indicated by names but by Roman numerals. Those subjects listed in this column, in the order as listed, were assigned the following numbers in his paper: XXI, XXVIII, XXXVI, XXIII, XXIX, XX, and XXXIX, respectively.

GENERAL CONSIDERATIONS. The general experience of the Nutrition Laboratory has been that with one and the same individual, when a relatively low pulse rate prevails, the metabolism is, in general, somewhat lower than when a relatively high pulse rate prevails. But this thesis deals only with the individual and not with the group. Hence from this standpoint alone no correlation between the average pulse rate of the group of Maya as a whole and their average metabolism is to be expected. Examination of the data for any one subject in our series shows that the

pulse rates for the most part are fairly uniform on the three different days. When material differences occur, there is occasionally a lower metabolism simultaneously with a lower pulse rate, but for the most part there is no correlation between these functions. Unless the heart rate may be taken as a direct measure of blood flow, obviously no correlation can be expected. It is conceivable, however, that with the Maya, as distinguished from most of the Whites, the volume output of the heart may vary greatly under different conditions. But it is clear that the heart rate and the total metabolism are not correlated in the individuals in this group. Indeed, of the 11 subjects having unusually low pulse rates, averaging 49 beats per minute or below, not one has a metabolism below the Harris-Benedict standard. All but one are above the prediction standard by 3.5 per cent or more, and five of them are more than 10 per cent above.

Had this high metabolism been noted in the case of a few Maya only, one might consider that it was but a chance observation. We believe that it has been established beyond question, however, that male Maya have a pronouncedly high metabolism and, in most instances, a low pulse rate. The reason for this high metabolism is difficult to understand. It has challenged attention from the time of Williams' first report, and the fact that Williams' observations were repeated by two later expeditions in the attempt to confirm or refute his measurements demonstrates that this finding (confirmed by all three expeditions) has real importance in the general study of racial metabolism. Dietary studies, particularly a study of the total nitrogen output per day, should be made with this most interesting people. A cursory survey of the food habits of the Maya leads to the belief that these men were on a distinctly protein-poor, non-stimulating diet. The factor of a protein-rich diet, therefore, and furthermore such factors as the bustle and drive typical of an active metropolitan center and extreme athletic prowess, which are known to stimulate metabolism, did not obtain under the conditions noted in Yucatan.

These Maya were living in a sub-tropical climate, under semi-primitive conditions. Climatological records for the years 1928 to 1931 show that the *minimum* temperatures in Chichen Itzá, when the experiments with the Maya during the second and third expeditions were made, were on the average as follows: January, 14.5°; February, 14.8°; March, 16.3°; and April, 18.2°C. In not a few instances during the third series of observations, temperatures at 6 a.m. of 8° to 10°C. were actually noted. Based upon experience in Western Cuba and his endocrinological studies, our colleague, Dr. Oscar Riddle[2] of the Department of Genetics of the Carnegie Institution of Washington, has emphasized the possibility that we have to

[2] We are very grateful to Doctor Riddle for his repeated, painstaking reviewing of this manuscript and for his keen, helpful criticisms.

deal here with a temporary adjustment of the thyroid to offset the effects of extremely cool, if not cold nights. The Maya sleeps inadequately protected by his meager clothing and his hammock against these cold nights. The majority of Williams' observations, however, were made in July, when the average *minimum* temperature is more nearly 20°C., and yet his July measurements show a definitely high metabolism. We are therefore convinced that in this high metabolism we are dealing primarily with a racial characteristic, although we have not as yet completely disposed of the possibility of a climatological factor.

The suggestion made in Williams' report that the metabolism is higher with the Indians having the purer Maya blood was challenged in the Shattuck paper. Since these two articles have appeared, a monograph has been published by Williams (9) in which his original assertion is still maintained. Our anthropometric studies convince us that Williams' thesis is definitely strengthened by our observations. We believe that our subjects were of an even purer Maya strain than his, which may in part, at least, account for the fact that the average percentage deviation in metabolism of our subjects is higher by 3 than Williams' average percentage deviation.

Just prior to the preparation of this present report there appeared another paper published in connection with the comprehensive program of the Nutrition Laboratory in the study of racial metabolism, namely, the account of the observations made by Prof. Eleanor D. Mason of the Women's Christian College at Madras, India, on the basal metabolism of native South Indian women (10). Fifty-four young women students (chiefly Tamils, Malayalis, and Telugus) served as subjects. The metabolism was observed in all but one instance on at least two days, comprising from 4 to 6 periods, and frequently many more. The pelidisi of these young women did not indicate an abnormal condition or state of undernutrition, and yet their metabolism on the average was 17 per cent below the Harris-Benedict predictions. It is of interest to compare this group of South Indian women with the group of male Maya, since they have such distinctly different metabolic levels. The group of 30 male Maya were found to have an average heat production of 1001 calories per square meter of body surface per 24 hours. Among the South Indian women a group of 27 Tamils had an average surface area, by the height-weight formula (7), of 1.39 sq. m. and an average 24-hour heat production of 753 calories per square meter of body surface. On this basis the metabolism of the male Maya is 33 per cent above that of the female Tamils.

At the time of the formulation of the surface-area law, no consideration was given to differences in sex and age, and certainly none to differences in race. Among these Maya and Tamils there is a slight difference in age, since the Maya average 27 years and the Tamils 21 years. But the most

important factors are sex and race. According to the Aub and Du Bois prediction standards, women are considered to have a metabolism 7 per cent lower than that of men. The experience of the Nutrition Laboratory leads us to believe that the difference in the heat production of men and women is somewhat greater than this (11). By no process of reasoning, however, can this difference of 33 per cent between the metabolism of the Tamils as a group and that of the Maya as a group be ascribed wholly to the influence of sex. In the earlier pronouncement of the surface-area law (12), values were given for the heat production of various animals per square meter of body surface, ranging from 1188 calories with the mouse to 917 calories with the rabbit. The metabolism of the mouse was 30 per cent higher than that of the rabbit. Thus, as is seen from the comparison of the metabolism of the Maya and the Tamils, the variability in the metabolism of humans is somewhat greater than it is within the group of various animals whose metabolism data were used for formulating the surface-area law. Furthermore, the animals were studied under conditions where thermic neutrality did not exist. With humans a condition of thermic neutrality does obtain and the basal metabolism is therefore, strictly speaking, more nearly the true basal. The fact should therefore not be lost sight of, particularly in the use of basal metabolism predictions and in basal metabolism studies, that of two groups of humans in thermic neutrality, one group may have a heat production per square meter of surface area 33 per cent above that of the other group. This difference can be attributed only in small part to differences in age and sex; it may be due partly to environment, but there is a good deal of evidence to indicate a racial effect.

SUMMARY

A third expedition to Yucatan confirms the findings of two earlier expeditions, that the male Maya have a high basal metabolism and a low pulse rate. A group of 30 male Maya had a heat production on the average 8 per cent above the prediction standards for Whites. Anthropometric measurements indicated that these Maya were well nourished and had a configuration not greatly different from that characteristic of Whites. The average pulse rate was 52 beats per minute. With one subject rates as low as 34 to 39 were noted, and with four others rates of about 45. The possibility of great intra-specific differences in metabolism is shown by the fact that the heat production of this Maya group was 33 per cent above the measured metabolism of a group of 27 South Indian women. This difference in metabolism can be attributed only in small part to the influence of age and sex and in somewhat larger part to the influence of environment, but the major part of the difference must be considered indicative of a real racial effect.

BIBLIOGRAPHY

(1) WILLIAMS, G. D. AND F. G. BENEDICT. This Journal, 1928, lxxxv, 634.
(2) SHATTUCK, G. C. AND F. G. BENEDICT. This Journal, 1931, xcvi, 518.
(3) BENEDICT, F. G. Boston Med. Surg. Journ., 1927, cxcvii, 1161; Ibid., Chinese Journ. Physiol., Report Series no. 1, 1928, p. 39; Ibid., Abderhalden's Handb. d. biolog. Arbeitsmethoden, 1929, Abt. iv, Teil 13, 1.
(4) PIRQUET, C. An outline of the Pirquet system of nutrition. Philadelphia and London, 1922, pp. 11–17 and 89–96.
(5) HARRIS, J. A. AND F. G. BENEDICT. Carnegie Inst. Wash. Pub. no. 279, 1919, pp. 40, 66, and 253 et seq.
(6) BENEDICT, F. G., W. R. MILES, P. ROTH AND H. M. SMITH. Carnegie Inst. Wash. Pub. no. 280, 1919, pp. 384 et seq.
(7) DU BOIS, D. AND E. F. DU BOIS. Arch. Intern. Med., 1916, xvii, 863.
(8) AUB, J. C. AND E. F. DU BOIS. Arch. Intern. Med., 1917, xix, 831.
(9) WILLIAMS, G. D. Maya-Spanish Crosses in Yucatan, Papers of Peabody Museum of American Archaeology and Ethnology, Harvard University, Cambridge, Mass., 1931, xiii, no. 1, pp. 94 et seq.
(10) MASON, E. D. AND F. G. BENEDICT. Indian Journ. Med. Research, 1931, xix, 75.
(11) MACLEOD, G., E. E. CROFTS AND F. G. BENEDICT. This Journal, 1925, lxxiii, 462; Ibid., Proc. Nat. Acad. Sci., 1925, xi, p. 343; F. G. BENEDICT. This Journal, 1928, lxxxv, 619.
(12) RUBNER, M. Die Gesetze des Energieverbrauchs bei der Ernährung. Leipzig, 1902, p. 282.

INSULIN IN TISSUES OTHER THAN THE PANCREAS

C. H. BEST, C. M. JEPHCOTT AND D. A. SCOTT

From the Department of Physiology and the School of Hygiene, University of Toronto

Received for publication January 4, 1932

A review of the literature, in which the distribution of insulin or insulin-like substances has been discussed, reveals the fact that numerous investigators have reported the presence of these active materials in a great variety of animal and vegetable tissues. With the procedure originally used to obtain insulin from the pancreas, insulin was not obtained from other tissues (Banting and Best, 1922). It was perhaps not surprising that the improved methods of extracting pancreatic insulin when applied to other animal tissues should apparently yield definite amounts of active material (Collip, 1923; Best, Scott and Banting, 1923; Ashby, 1923; Baker, Dickens and Dodds, 1924; Best, Smith and Scott, 1924; Ivy and Fisher, 1924; Lundberg, 1924; Brugsch and Horsters, 1924 and 1930; Nothmann, 1925; Cori, 1925; Vincent, Dodds and Dickens, 1925; Penau and Simonnet, 1925; Hoshi, 1926; Cramer, Dickens and Dodds, 1926; Redenbaugh, Ivy and Koppanyi, 1926; Shikinami, 1928). Certain points in these reports on the distribution of insulin stand out very prominently. It is surprising that insulin should be reported to be present in diabetic tissues, (Best, Smith and Scott, 1924; Baker, Dickens and Dodds, 1924; Nothmann, 1925; Pollak, 1926) and in relatively large amounts in the zymogenous tissue of teleosteal fishes (Vincent, Dodds and Dickens, 1925) and in spleens of normal rats (Cramer, Dickens and Dodds, 1926). Furthermore, the urinary secretion of very large amounts of active material after the intravenous or oral administration of insulin, or after its introduction through an intestinal fistula (Fisher and Noble, 1923) and the peculiar behaviour of "endogenous" and "exogenous" insulin (Brugsch and Horsters, 1924, 1930), are very odd phenomena.

The apparently wide distribution of insulin-like material in the animal kingdom led Collip to suggest that this material might be present wherever glycogen was formed. The active material was, however, later thought to be present in extracts of plants, in the tissues in which no glycogen can be detected (Collip, 1923). Collip also encountered a strange phenomenon of "animal passage" of the hypoglycemia-producing principle. Later he suggested (Collip, 1927) that the passage phenomenon was probably attributable to a "living agent." It is obviously impossible to decide from

the evidence at present available how much of the delayed hypoglycemia observed after the injection of extracts from vegetable sources was due to a chemical substance, and how much to the effects of the "living agent" which might have been present in the extracts. The evidence suggests that most of the changes observed by Collip were due to a contaminating "living agent." It must be borne in mind, however, that large doses of pancreatic insulin may produce a prolonged hypoglycemia after a preliminary period in which the blood sugar is not lowered. It is perhaps significant that some of the workers with plant extracts secured prompt hypoglycemia (Dubin and Corbitt, 1923; Best and Scott, 1923; Fisher and McKinley, 1924; and others). Collip (1923) secured only delayed effects from yeast extracts, while Hutchinson, Smith and Winter (1923) secured more rapid effects comparable to those produced by pancreatic insulin. In Collip's work on extract from clams, hypoglycemic convulsions were observed in rabbits within six hours. The possible rôle of pancreatic insulin in the production of some of these results will be clearer when the results we have recently obtained have been discussed.

There are a number of reasons why the results of the early work on the distribution of insulin and insulin-like substances are of no significance from the quantitative point of view. It was not then fully appreciated that some rabbits may show a definite fall in blood sugar over the experimental period, and for this reason the results based on one animal are not satisfactory. A standard of comparison was not used in the assaying of insulin in most of the early work. In a number of the papers which have appeared on the subject of the distribution of insulin, no convincing evidence is to be found that any hypoglycemia-producing substance was present in the extracts which were reported to yield positive results. When we add to these factors the possibility of the presence of a living agent which may cause hypoglycemia, the possibility that non-specific toxic materials, which may produce liver damage and delayed hypoglycemia, may have been present, and the possibility of contamination of solutions by pancreatic insulin, it becomes apparent that the whole question of the distribution of insulin and insulin-like substances must be reopened, and that many, if not all, of the results obtained up to the present time be discarded.

In the previous work several investigators have stated that they were able quantitatively to recover insulin added to pancreas or other tissues. Few, if any, of these results will, in the light of the present knowledge, stand critical analysis. In most cases one of the well known methods for making pancreatic insulin was used. In work of this kind it would be preferable to have available a method that would yield optimal results when applied to pancreatic tissue. With this point in mind, one of us (Jephcott, 1931) has thoroughly explored the conditions under which maximal yields of

insulin in a form in which it can be accurately assayed are obtainable from pancreatic tissue. The details of the procedure finally adopted are as follows: perfectly fresh glands are finely minced, and after thorough stirring 25 gram samples are quickly weighed and transferred to a flask containing 75 cc. of 95 per cent ethyl alcohol, 25 cc. of water, and 1.5 cc. of concentrated hydrochloric acid. Extraction is carried out in a mechanical shaker immersed in a water bath which is kept at a temperature of 36°C. After two hours the mixture is filtered through a double thickness of gauze and the residue is pressed as dry as possible. A second extraction, similar to the first, is then made. The two filtrates are combined, made just alkaline to litmus with ammonia, filtered through a Buchner funnel and re-acidified to pH2 with hydrochloric acid. The alcohol is distilled off under vacuum at about 35°C. The residue is transferred to a 250 cc. measuring flask and made up to volume with water. When desirable the active material may be salted out with ammonium sulphate or sodium chloride without appreciable loss. The assay on white mice and rabbits is conducted as soon as possible after the extract has been prepared. The assays are made by comparison of the effect of the unknown solution with that of standard insulin. After the potency of the solution is determined approximately, from thirty to sixty white mice and several rabbits are used for each test. When the procedure outlined above is used to extract insulin from beef pancreas, average yields of approximately 3000 units per kilogram may be consistently obtained. We believe that yields of 3500–4000 units per kilogram represent the maximal amounts of active material which can be secured by the methods available at present from beef pancreas collected in Toronto during the summer months. Higher yields reported by other workers are open to question since the methods of assay then used cannot now be considered adequate. The yields of insulin from the pancreas of various species have been determined by one of us (C. M. J.) and will shortly be reported.

It might be expected that no difficulty would be encountered in recovering added insulin with a method such as we have outlined. Such, however, is not the case. Purified insulin or the crystalline product cannot consistently be recovered quantitatively when the hydrochloric acid procedure outlined above is used. The results of numerous experiments demonstrate that an average recovery of more than 60 per cent of the added material is not usually secured. When sulphuric acid is used instead of hydrochloric the recovery is not significantly improved. Lower yields of insulin from pancreas are always obtained when sulphuric acid is used.

It can be shown, however, that crude insulin withstands this treatment much better than the purer material (Jephcott, 1931). One of the factors affecting the destruction of the purified insulin may be the length of time

it is exposed to the alcohol and acid. Even when the tissue is extracted rapidly, however, a great deal of the added material may be lost. Insulin prepared by different procedures varies considerably in its stability under these circumstances.

The situation is, therefore, that the method which gives the best yield of insulin from pancreas does not consistently permit more than a partial recovery of purified insulin added to pancreas or to other tissues. When the hydrochloric acid procedure is used to investigate the insulin content of tissues other than the pancreas, negative results might conceivably mean that small amounts of insulin were present but were destroyed by the treatment. If, however, the hydrochloric and sulphuric acid extractions and various methods of purification are carefully applied and negative results are consistently obtained, the suggestion that demonstrable amounts of insulin or insulin-like materials are not obtainable from other tissues would be supported.

The results of the application of these procedures to vegetable tissues,

TABLE 1

TISSUE	WEIGHT	METHOD	MOUSE TEST	RABBIT TEST
	grams			
Onions	1,100	Alcoholic HCl	Negative	Negative
Beet roots	1,400	Alcoholic HCl	Negative	Negative
Beet roots	1,100	Alcoholic H_2SO_4	Negative	Negative
Beet roots	1,800	Alcoholic HCl	Negative	Negative

normal beef and dog tissues, to urine and to diabetic dog tissues will be briefly reported and discussed.

Vegetable tissues. It is unnecessary to review in detail the numerous reports on the presence of insulin-like substances in plant tissues (Collip, 1923 a, b, c, d, e; Funk and Corbitt, 1923; Dubin and Corbitt, 1923; Winter and Smith, 1923 and 1925; Hutchinson, Smith and Winter, 1923; Best and Scott, 1923; Gottschalk, 1924; Eisler and Portheim, 1924; Glaser and Wittner, 1924; Fisher and McKinley, 1924; Simola, 1927; Shikinami, 1928; Kaufmann, 1928, etc.). The results of these studies may be divided into three groups; *a*, in which there is no convincing evidence of the presence of any active principle; *b* in which there is proof that a delayed hypoglycemia occurs, and *c*, in which the hypoglycemia appears more promptly. If we make the obvious inference from Collip's suggestion that a living agent may be responsible for the "animal passage" phenomenon, that it might also account for the delayed hypoglycemia observed after the subcutaneous injection of vegetable extracts, and admit the possibility that contamination of the plant extracts by pancreatic insulin may be responsible for the more rapid effects, there would be no evidence suggesting an insu-

TABLE 2

TISSUE	AMOUNT	METHOD OF EXTRACTION	METHOD OF ASSAY	RESULT*
Arterial blood (dog); no general anesthetic	160 cc. serum	Alcoholic H_2SO_4	Mouse and rabbit	Negative
	205 cc. cells	Alcoholic H_2SO_4	Mouse and rabbit	Negative
Arterial blood (dog); no general anesthetic	540 cc. defibrinated blood	Benzoic acid	Mouse and rabbit	Negative
Splenic vein blood (dog); amytal anesthesia	57 cc.	Benzoic acid	Mouse	Negative
Dog's liver	230 grams	Alcoholic HCl	Mouse	Negative (toxic effects)
Dog's liver	100 grams	Benzoic acid	Mouse and rabbit	Negative
Dog's liver	150 grams	Picric acid	Mouse and rabbit	Negative
Dog's liver	75 grams	Fisher's method	Mouse and rabbit	Negative
Sheep liver	60 grams	Alcoholic HCl	Mouse and rabbit	Negative (toxic effects)
Sheep liver	100 grams	Picric acid	Mouse and rabbit	Negative
Beef liver	10 grams	Alcoholic HCl	Mouse	Negative
Dog's muscle	100 grams	Benzoic acid	Mouse and rabbit	Negative
Beef salivary glands	100 grams	Alcoholic HCl	Mouse and rabbit	Negative
Beef salivary glands	500 grams	Picric acid	Mouse and rabbit	Negative
Dog's heart	50 grams	Picric acid	Mouse and rabbit	Negative
Dog's heart	38 grams	Fisher's method	Mouse	Negative
Dog's kidney	47 grams	Alcoholic HCl	Mouse	Negative
Dog's spleen	35 grams	Alcoholic HCl	Mouse	Negative
Beef thymus	800 grams	Benzoic acid	Mouse and rabbit	Negative

* The possibility that the very potent but labile form of insulin reported by Dingemanse (Arch. Exp. Path. Pharm. 128, 44, 1928) and more recently observed by Dirscherl (Hoppe-Seyler's Ztschr. f. Physiol. Chemie, 202, 116, 1931) may have been responsible for any of the positive results previously obtained can, in our opinion, be regarded as extremely faint.

lin-like substance (glucokinin) in vegetable tissues. Collip (1927) apparently does not consider that the initial hypoglycemia produced by the vegetable extract is attributable to the same factors which cause the "animal passage" hypoglycemia.

When vegetable tissues were extracted by the procedure most successfully used to obtain pancreatic insulin, negative results were secured.

The extracts made from beet roots produced very toxic effects in several of the rabbits.

While sufficient experiments have not been performed to justify the conclusion that an insulin-like substance (glucokinin) is not present in plant tissues, it may be stated that in light of the recent findings the previous reports cannot be accepted at their face value. (The substances which may be extracted from members of the myrtle family, and which are reported to produce an antidiabetic effect when given by mouth, are not considered in this discussion.)

Normal animal tissues. A great many experiments have been conducted in this part of our work. A limited number of typical results will be reported. In addition to the methods we have discussed above, the exact procedures advocated by Fisher and by Baker, Dickens and Dodds and by others have been used.

Diabetic tissues. The failure to demonstrate insulin in normal tissues other than the pancreas made it extremely unlikely that any antidiabetic principle would be found in diabetic organs. We have taken advantage of an opportunity to secure diabetic tissues, however, and these have been analyzed by two methods (sulphuric and hydrochloric acid extraction) in an attempt to secure insulin. Liver, skeletal muscle and heart muscle were removed from a completely depancreatized dog which had received no insulin for 5 days. The results of the assay of the extracts prepared were completely negative. It does not follow from these results that the pancreas of a patient dying in diabetic coma contains no insulin, but a reinvestigation of this subject is perhaps necessary.

Insulin in urine. A hypoglycemia-producing substance has been reported to be present in urine by several groups of workers (Best, Smith and Scott, 1924; Kozuka, 1927; Partos, 1929; Brugsch and Horsters, 1930). This aspect of the subject of the distribution of insulin has been particularly developed by Partos and by Brugsch and Horsters. Recently Laurence, Modders and Millar (1930) have reported that they are unable to confirm the results obtained by these investigators.

We have reinvestigated this field and have been unable to secure demonstrable amounts of insulin from normal dog or human urine.

Recovery of injected insulin. The urinary excretion of injected insulin (Fisher and Noble, 1923) needs further comment. These investigators reported that large amounts of insulin could be recovered from the urine

of normal dogs after the oral or intravenous administration of the active material. Soon after this work was published R. G. Smith in our laboratory attempted to repeat it. In his first experiments he was apparently successful but later in numerous experiments was unable to detect more than traces in the urine even after the oral or intravenous administration of enormous doses to dogs. So many possible variables were uncontrolled in these experiments that the results were not reported at the time. Quite recently we have repeated certain of these experiments with the following results. When an insulin solution is administered intravenously to a dog under amytal anesthesia at such a rate that 200 units are given in the first two hours it is found that less than 10 per cent of the injected insulin can be recovered from the urine voided during the four hour experiment. Saline was given during the last two hours of the experiment. The urine was acidified and immediately tested for insulin by injection directly into mice and rabbits. No insulin could be recovered by the benzoic acid method from liver, kidney, muscle or heart tissues which were removed at the end of the experiment. These results demonstrate that, although most of the injected insulin was lost, a small but definite amount can be recovered from the urine under the conditions of these experiments. In a few experiments in which large amounts of insulin were administered by mouth to normal dogs, none was recovered in the urine.

Discussion. It is necessary to define what is meant by negative result in the assay of solutions for their insulin content by the mouse and rabbit methods. As it is not advisable to administer more than 0.5 cc. of solution to a mouse, the volume of all extracts was kept as small as possible. From 0.25 to 0.50 cc. was injected. When no symptoms attributable to the presence of insulin are observed in any of the injected animals it is safe to conclude that less than 1/100 unit of insulin is present. As approximately 25 cc. of solution were obtained from 100 grams of tissue a negative result indicates that less than $\frac{1}{2}$ unit was present in the extract from this amount of material. Mice may occasionally exhibit convulsions which resemble those produced by insulin when tissue extracts which do not contain demonstrable amounts of this active material are injected. It is sometimes difficult to decide whether or not the administration of dextrose solution improves their condition. Repetition of tests, the results of which are difficult to interpret, and comparison with the results of the rabbit assay, however, always enable an investigator to make a correct interpretation of these doubtful results. Rabbits are injected subcutaneously with from 10 to 15 cc. of solution. A blood sugar lowering of from 30 to 40 mgm. per cent when the normal value is high (0.13 to 0.15 per cent) is not proof of the presence of insulin. It is inadvisable to conclude that insulin is present if very large doses of the extract under test do not

lower the blood sugar of some of the test rabbits fairly rapidly to the convulsive level.

One investigator (F. A. Calderone, who was working under the direction of Prof. G. B. Wallace) has informed us that he has been unable to confirm the results of the workers who reported the presence of insulin in blood (Best, Scott and Banting; Nothmann; Hoshi) but as far as we know there have been no published reports to this effect. Until we had secured the negative results reported above, we were inclined to attribute these apparent difficulties to the same factors which, in the early stages of the work on insulin, made it impossible consistently to obtain active material from pancreatic tissue. The demonstration of insulin in blood by physiological methods (Zunz and La Barre, 1927–29) seems to be convincing. The real situation very likely is that suggested by the observations of Kepinow and Petit-Dutaillis (1927) and of Heymans (1927). Insulin added to blood kept at body temperature was found to disappear very rapidly. In the light of our recent observations we believe that there is no evidence that any tissue other than pancreatic either produces or stores insulin. It is, of course, possible that methods will be developed which will permit the recognition of the minute amounts of insulin that one would expect to be present. Although we have not attempted to repeat in detail the extensive work of Brugsch and Horsters, from the results of which they conclude that injected insulin liberates endogenous insulin, etc., etc., we suggest that their results may be different if the experiments are repeated, using the precautions suggested by our experimental results.

A great deal of space might be utilized in the further discussion of the results which we have been unable to confirm. Many of these considered to be positive are obviously attributable to what we now know to be faulty interpretations of the results of the assays. The possibility exists that a contaminating living agent may have been responsible for the positive results in some cases. There is no doubt, however, that the active material found to be present in extracts of these various tissues by several groups of workers was insulin. It is perhaps significant that in Toronto, and in Collip's laboratory, large amounts of insulin were being prepared from pancreas. In Dodds' and Dickens' laboratory, work on the preparation of insulin was also in progress. In the laboratories where Fisher and Ashby, and Lundberg were studying, interest in the preparation and purification of insulin was being taken at the time the work referred to above was done. Although insulin clings tenaciously to laboratory glassware, etc., the usual cleaning procedures carefully applied are sufficient to remove the last traces. If contamination of solutions did occur, it is obviously impossible to make any rational suggestion concerning the mechanism.

It is regrettable that a great deal of what appears to be useless work has been done in this field. The results reported in this paper, in our opinion,

serve to remove from serious consideration many reports which made it difficult to accept the pancreas as the only source and the only important storehouse of insulin in the animal body.

SUMMARY

Using a method of extraction which yields maximal amounts of insulin from pancreas, we have been unable to demonstrate the presence of an antidiabetic substance in other normal tissues from the dog or ox or in the tissues of diabetic dogs. Positive results previously reported we believe can be attributed to one or more of several factors, each of which is discussed in this communication. Certain experimental results which have indicated a wide distribution of an insulin-like substance in the plant kingdom have not been confirmed.

We have had the opportunity of discussing these results with Prof. E. C. Dodds and Prof. A. C. Ivy. These investigators have repeated certain of the experiments performed in their laboratories, the results of which suggested that insulin or an insulin-like substance could be obtained from mammalian tissues other than the pancreas. The results of these recent experiments confirm the negative findings reported in this paper.

BIBLIOGRAPHY

Ashby, J. S. 1923. This Journal, lxvii, 77.
Baker, S. L., F. Dickens and E. C. Dodds. 1924. Brit. Journ. Exp. Path., v, 327.
Banting, F. G. and C. H. Best. 1922. Journ. Lab. Clin. Med., vii, 464.
Best, C. H. and D. A. Scott. 1923. Trans. Roy. Soc. Canada, xvii, sec. v, 87.
1923. Journ. Amer. Med. Assoc., lxxxi, 382.
1923. Journ. Metab. Res., iii, 177.
Best, C. H., D. A. Scott and F. G. Banting. 1923. Trans. Roy. Soc. Canada, xvii, sec. v, 81.
Best, C. H., R. G. Smith and D. A. Scott. 1924. Journ. Biol. Chem., lix, xxx.
1924. This Journal, lxviii, 161.
Brugsch, T. and H. Horsters. 1924. Biochem. Zeitschr., cxlvii, 150.
Brugsch, H. and H. Horsters. 1930. Arch. exp. Path. u. Pharm., cxlviii, 295.
Collip, J. B. 1923. Proc. Soc. Exp. Biol. and Med., xx, 321.
1923. Nature, cxi, 571.
1923. Trans. Roy. Soc. Canada, xvii, sec. v, 39.
1923. Trans. Roy. Soc. Canada, xvii, sec. v, 45.
1923. This Journal, lxiii, 391.
1923. Journ. Biol. Chem., lv, xxxix.
1923. Journ. Biol. Chem., lvi, 513.
1923. Journ. Biol. Chem., lvii, 65.
1923. Journ. Biol. Chem., lviii, 163.
1927. Proc. Soc. Exp. Biol. and Med., xxiv, 731.
Cori, G. T. 1925. Journ. Cancer Res., ix, 408.
1925. This Journal, lxxi, 708.
Cramer, W., F. Dickens and E. C. Dodds. 1926. Brit. Journ. Exp. Path., vii, 299.

DUBIN, H. E. AND H. B. CORBITT. 1923. Proc. Soc. Exp. Biol. and Med., xxi, 16.
EISLER, M. AND L. PORTHEIM. 1924. Biochem. Zeitschr., cxlviii, 566.
FISHER, N. F. AND E. B. MCKINLEY. 1924. Proc. Soc. Exp. Biol. and Med., xxi, 248.
FISHER, N. F. AND B. E. NOBLE. 1923. This Journal, lxvii, 72.
FUNK, C. AND H. B. CORBITT. 1923. Proc. Soc. Exp. Biol. and Med., xx, 422.
GLASER, E. AND L. WITTNER. 1924. Biochem. Zeitschr., cli, 279.
GOTTSCHALK, A. 1924. Deutsch. Med. Wochenschr., l, 538.
HEYMANS, J. F. AND C. HEYMANS. 1927. Compt. rend. Soc. Biol., xcvi, 719.
HOSHI, T. 1926. Tohoku Journ. Exp. Med., vii, 442, 446.
HUTCHINSON, H. B., W. SMITH AND L. B. WINTER. 1923. Biochem. Journ., xvii, 683, 764.
IVY, A. C. AND N. F. FISHER. 1924. This Journal, lxvii, 445.
JEPHCOTT, C. M. 1931. Trans. Roy. Soc. Canadà, xxv, sec. v, 183.
KAUFMANN, E. 1927. Zeitschr. ges. exp. Med., lv, 1.
1928. Zeitschr. ges. exp. Med., lx, 285, lxii, 147, 154, 739.
KEPINOW, L. AND S. L. PETIT-DUTAILLIS. 1927. Compt. rend. Soc. Biol., xcvi, 371, xcvii, 25.
1929. Arch. Intern. Physiol., xxxi, 310.
KOZUKA, K. 1927. Tohoku Journ. Exp. Med., ix, 130.
LA BARRE, J. 1927. Compt. rend. Soc. Biol., xcvi, 193, 196.
1927. Arch. Intern. Physiol., xxix, 238; 257.
1928. Compt. rend. Soc. Biol., xcviii, 859.
LAURENCE, R. D., K. MODDERS AND H. R. MILLAR. 1930. Brit. Journ. Exp. Path., xi, 117.
LUNDBERG, E. 1924. Compt. rend. Soc. Biol., xci, 418.
NOTHMANN, M. 1925. Arch. exp. Path. u. Pharm., cviii, 1.
1926. Klin. Wochenschr., v, 297.
PARTOS, A. 1929. Pflüger's Arch., ccxxi, 562.
PENAU, H. AND H. SIMONNET. 1925. Bull. soc. chim. biol., vii, 17.
POLLAK, L. 1926. Arch. exp. Path. u. Pharm., cxvi, 15.
REDENBAUGH, H. E., A. C. IVY AND T. KOPPANYI. 1926. Proc. Soc. Exp. Biol. and Med., xxiii, 756.
SHIKINAMI, Y. 1928. Tohoku Journ. Exp. Med., x, 1560.
SIMOLA, P. E. 1927. Ann. Acad. Sci. Fenn., xxix.
VINCENT, S., E. C. DODDS AND F. DICKENS. 1925. Quart. Journ. Exp. Physiol., xv, 313.
WINTER, L. B. AND W. SMITH. 1923. Journ. Physiol., lvii, xl.
1925. Journ. Physiol., lx, v.
ZUNZ, E. AND J. LA BARRE. 1927. Compt. rend. Soc. Biol., xcvi, 421, 708, 710, 1045.
1927. Arch. Intern. Physiol., xxix, 265.
1928. Compt. rend. Soc. Biol., xcix, 335.
1929. Arch. Intern. Physiol., xxxi, 20.

THE EFFECTS OF SYMPATHETIC DENERVATION OF MAMMALIAN TISSUES DURING THE PERIOD OF POST-NATAL GROWTH

S. S. TOWER

From the Department of Anatomy, the Johns Hopkins University

Received for publication February 1, 1932

A series of studies, to which the author has contributed (1931), has sought in vain, in the adult of mammalian species, for a trophic influence exerted by the sympathetic nervous system over non-nervous tissues in general. In particular, skeletal muscle shows no structural alteration following sympathetic denervation. However, the life which an adult animal leads in a laboratory—confined in a cage, fed and protected,—provides perhaps, an inadequate test for a tenuous factor in trophic control such as the sympathetic nervous system has been thought to supply. Cannon (1930) and his students found, indeed, that their completely de-sympathectomized animals could survive only under such carefully regulated conditions. However, it is common experience that the same life in a laboratory presents grave difficulties to the young of a species. Therefore, development of young animals under these conditions seemed to offer a most stringent trial of the importance of the sympathetic nervous system in nutritional or metabolic regulation of non-nervous tissues. With this in mind, the present study was undertaken.

MATERIAL: METHODS. A series of kittens was operated on between the seventh and tenth days of life. Two operations were performed. In one group of animals, the right stellate ganglion and thoracic sympathetic trunk were removed down to the 4th thoracic interspace. Of these animals, six survived. In a second group, in addition to the operation just described, the entire right cervical sympathetic chain was also resected, ganglia, trunk and ansa subclavia. Of these, four survived.

The members of each group were killed at the expiration of allotted times; six weeks, three months, six months and a year. The two extra animals of the first group were kept for the longer periods,—six and twelve months. The animals were killed with ether, the blood vessels washed out with physiological salt solution, portions of muscle removed to assorted fixatives, and the entire animal injected with 80 per cent alcohol.

At autopsy the size and development of corresponding structures on the two sides of the body were compared. Specimens were removed and weighed. Finally, histological preparations were made, utilizing the

Bielschowsky silver technique for muscle, Einarson's (1932) new gallocyanin technique for sympathetic ganglia, the Ranson-Cajal technique for sympathetic nerve trunks, and iron hematoxylin for all other tissues. Ranvier's gold chloride technique was also applied to muscle, for the study of the perivascular nerve plexuses.

Observations. The search for a so-called trophic influence exercised by the sympathetic nervous system on non-nervous tissues during the crucial period of growth and maturing of the animal, proved entirely fruitless. In the living animal, the development of the fore limbs, and indeed, of the entire fore half of the body, was quite symmetrical. The muscles, in bulk and strength; the bones, in size and hardness; the skin, hair,—all showed no effect of the prolonged sympathetic denervation. Only the complete and permanent loss of sudomotor and vasomotor function on the side of operation, and the Horner's syndrome, gave evidence of the lesion, and index to the state of repair.

The weights of specimens taken at autopsy from the four animals with complete thoraco-cervical sympathectomy, also showed negligible differences between the operated and normal sides. These specimens comprised the halves of the brain, the palmaris longus and extensor digitorum lateralis muscles, and the lateral lobes of the thyroid gland. One defect only was noted at autopsy, other than the absence of the sympathetic tissue removed at operation. The right superior cervical ganglion was consistently smaller than the left in the six cats in which it remained. (A series of six normal cats established the practical equality in size of these two ganglia, ordinarily.)

Examination of the microscopic preparations confirmed the gross observations. The Vth interosseous muscles were very carefully compared. But neither size of the muscle fibers, nor any characteristic such as striation, nucleation, staining reaction or granulation, betrayed the presence in the right muscles of a lesion of any sort. The smaller intra-muscular arteries and arterioles, which are supposed to be particularly under the influence of sympathetic innervation, were thoroughly inspected. Yet even they showed no effect of presumably complete denervation, other than the absence of perivascular non-medullated nerve fibers. The thyroid glands, finally, also gave no indication, by size of follicle, character of epithelium, quantity or staining quality of colloid, of a different functional state on the side of lesion and on the normal side.

Uniformly then, the non-nervous tissues examined, failed to react to, or compensated entirely for sympathetic denervation. Both grossly and microscopically, they had attained normal size and form quite without the influence of sympathetic innervation. That the tissues examined were indeed sympathetically denervated, that there had been no regeneration of preganglionic fibers to take over the work of the completely excised

postganglion neurone, nor diversion of postganglionic neurones from adjacent regions to supply the denervated area, there is abundant evidence. For the fore limb, first, careful dissection at autopsy disclosed no gross structures open to interpretation as sympathetic fibers passing to the brachial plexus. In agreement with the autopsy findings, sudomotor and vasomotor functions never returned to the paw-pad. Moreover, when the methods applied by Tower and Richter (1931, 32) to the study of sympathetic regeneration were used on the group of animals surviving operation a year, the skin-resistance of the right fore paw-pad was found to be in excess of the normal maximum for a cat, and the skin-action-currents (galvanic skin response), which are the most delicate indicator of sudomotor innervation, were totally wanting. Finally, as previously recorded, the blood vessels, whenever examined, were found to be depleted of non-myelinated nerve fibers on the side of lesion. On the other hand, the gross form of the ansa subclavia was more or less restored in all ten animals, by strands passing from the first two thoracic nerves. In the animals with inferior and superior cervical ganglia intact, these strands contained a great many nerve fibers. Especially was this the case six months or a year after operation, when functional recovery in the eye was far advanced. In the four animals with complete cervical sympathectomy, however, sections of these strands just central to their point of junction with the vagus, showed the structure to be largely connective tissue, containing only a very few tortuous nerve fibers. And in these animals, after a certain recovery of tone during the first month, Horner's syndrome was static. Here, most clearly, there was no functional regeneration or preganglionic fibers down the postganglionic path, nor crossing over of fibers from the normal side to supply the denervated region.

Although the non-nervous tissues affected by the lesion showed no effect of prolonged sympathetic denervation, the cervical sympathetic ganglia, superior and inferior, cut off from the central nervous system for a variable time by resection of the stellate ganglion, showed most marked reactions. In the inferior cervical ganglion the reaction took the form of an apparent slight hypertrophy, both gross and microscopic. There was an increase in the number of cells of the types normal to the ganglion. However, this ganglion varies exceedingly in size, being in some animals, microscopic, in others grossly well developed. Hence, little importance can be attached to observations in so small a series as six cats. In the superior cervical ganglion a consistent small gross size of the right ganglion was reflected microscopically in a greatly diminished number of nerve cells. The cells also averaged somewhat smaller and the connective tissue elements showed an increase, perhaps only relative. The reduction in number and size of the cells seemed to affect all the range of sizes and types in proportion to their normal representation. For example, the very large cells

(40 $\mu \times 70\ \mu$), which appear only after the first half-year of life, were present on the two sides in proportion, but ran a trifle larger on the normal side. A more conspicuous difference between the cells of normal and decentralized ganglia than was afforded by size or form, was the noticeably larger size of the nuclei and their predominantly eccentric or polar position, on the size of lesion. Furthermore, the cytoplasm of these cells appeared to shrink in fixation excessively, a characteristic reported by Sternschein (1922). However, the chromidial substance of nucleus and cytoplasm showed no disturbance on the side of lesion. Nor were pigment granules, a normal cytoplasmic constituent of many sympathetic cells, increased in amount as Graupner has described (1898) for conditions of atrophy of sympathetic ganglia.

Conclusive evidence to indicate the cause of the diminished cell count on the side of operation was wanting. Of cellular degeneration there was no sign, even in the specimen taken six weeks after operation. Since the operation decentralizing the superior cervical ganglion was performed before the cervical sympathetic had assumed function (Horner's syndrome develops between the second and third weeks of life) and probably before the complement of cells has been fully made up, and since, moreover, the differences between the right and left ganglia were the greater, the longer the time elapsing after operation, it seems likely that the deficiency in the decentralized ganglia was a matter rather of incomplete development than of degeneration. On the other hand, resumption of function by the superior cervical ganglion did not correct the defect. Recovery from pupillary paralysis began in the sixth to eighth week, reached the stage of pupillary equality generally by the sixth to eighth month, after which a condition of over-dilatation supervened. Yet, the greatest differences between right and left ganglia were found in the animals sacrificed after a year, at which time the eye on the side of operation had been in a state of sympathetic over-action for months.

Conclusions. From the evidence presented, certain conclusions may be drawn as to the activities of the sympathetic nervous system. First, during the taxing period of development of the new-born into the adult, the influence of the sympathetic nervous system is not necessary for non-nervous tissues, in general, to attain normal size and form. At any time during the first year of life many such tissues, sympathetically denervated, are quite indistinguishable from the normal. This conforms with histological evidence the observations of gross form and size, reported by Cannon (1930) for kittens, and by McCullogh, McFadden and Milroy (1930) for puppies. Second, the evidence shows that even in the young animal, when presumably the impediment offered in distance and density of structure is minimum, preganglionic neurones do not replace the post-

ganglionic, although they are themselves growing rapidly and re-establishing their own connections across considerable gaps. Moreover, the neurones of the opposite intact side, or of levels below the lesion, are not mobilized to mend a sympathetic defect. Finally the evidence indicates that the influence of the sympathetic outflow from the spinal cord is essential to the proper development of the peripheral sympathetic ganglia. Contrary to the report of Anderson (1902), decentralized sympathetic ganglia fail to attain the gross size of normal ganglia, and their cell complement is less. The reduction in numbers of cells, and in their size, affects all elements of the cell population equally. No two groups of cells such as Dogiel (1896) has described, can be discriminated,—the one perhaps sensory and stimulated from the periphery, or, more likely, responding to intraganglionic stimuli, humoral or reflex, and preserved following decentralization; the other, dependent both for function and for existence entirely on the central nervous system. That a sympathetic ganglion and its connections exercise some variety of control over peripheral structures, in particular over smooth muscle, there is ample proof (Tower and Richter, 1932). But of a special type of cell serving such functions, the present study affords no indication.

SUMMARY

A series of kittens was operated on, between the seventh and tenth days of life, for removal of a greater or lesser portion of the right cervicothoracic sympathetic chain. The animals were allowed to survive a fixed period up to one year. During this time growth and development were carefully studied. At autopsy, corresponding normal and sympathetically denervated organs were compared and weighed. Finally, histological preparations were made and studied.

In the non-nervous tissues examined, sympathetic denervation was without appreciable sequel. Gross specimens of bone and muscle, of brain and thyroid gland from the side of operation and the normal, were quite alike in size, weight and form. Microscopically also, the specimens from the two sides were indistinguishable. The sympathetic ganglia cut off from the central nervous system showed, on the other hand, marked deficiency in gross size and microscopically, in cell content. The cells likewise showed certain morphological peculiarities.

The conclusions drawn were: 1, that sympathetic innervation is not essential to the growth or maintenance of non-nervous tissue in general; 2, that preganglionic fibers do not replace postganglionic, even in the very young animal, nor do sympathetic neurones deviate from their proper course to repair a defect; 3, that decentralization of sympathetic ganglia hinders the development of all postganglionic neurones so affected.

BIBLIOGRAPHY

ANDERSON, H. K. 1902. Journ. Physiol., xxviii, 499.
BACQ, M. 1930. This Journal, xlv, 610.
CANNON, W. B. 1930. Lancet, May 24, 1109.
DOGIEL, A. S. 1896. Anat. Anz., xi, 679.
EINARSON, L. 1932. Amer. Journ. Path., May (in press).
GRAUPNER, R. 1898. Beitr. z. path. Anat. u. allg. Path., xxiv, 255.
MCCULLOGH, G. P., G. D. MCFADDEN AND T. H. MILROY. 1930. Journ. Physiol., lxix, 353.
STERNSCHEIN, E. 1922. Arb. a. d. Neurol. Inst., Wien., xxiii, 155.
TOWER, S. S. 1931. Bull. Johns Hopkins Hosp., xlviii, 115.
TOWER, S. S. AND C. P. RICHTER. 1931. Arch. Neurol. and Psych., xxvi, 485. 1932. (In press.)

THE EXCRETION OF URINE IN THE DOG

III. The Use of Non-metabolized Sugars in the Measurement of the Glomerular Filtrate

NORMAN JOLLIFFE, JAMES A. SHANNON AND HOMER W. SMITH

From the Department of Physiology, University and Bellevue Hospital Medical College, New York City

Received for publication December 29, 1931

No substance is known to be present normally in the plasma and urine of vertebrates which can be safely used to evaluate the quantity of glomerular filtrate under physiological conditions. Creatinine of exogenous origin has been recommended for this purpose by Rehberg, (1926) but when it is recognized that this substance is secreted by the renal tubules of the lower vertebrates (Marshall and Grafflin, 1932; Clarke and Smith, 1932) and that it is not present normally in significant quantities in the blood of mammals (Behre and Benedict, 1922; Gaebler and Keltch, 1928; Gaebler, 1930) its use is open to question.

We have sought for a foreign substance, practically and theoretically suitable for this purpose, among several groups of organic and inorganic compounds and have concluded that the non-metabolized sugars are satisfactory.

The properties which we believe such a substance should possess are as follows:

1. It must be determinable in plasma and urine with quantitative accuracy.
2. It must be non-toxic and it must exert no local stimulating or depressing action upon the kidney.
3. It must be completely filtrable from plasma.
4. It must not be secreted by the renal tubules.
5. It must not be reabsorbed by the renal tubules.

The choice of sugars for this purpose was suggested by the recent observations of Marshall (1930) that glucose is excreted by glomerular, but not by aglomerular fishes. On broad principles we relate this circumstance to the fact that, being a food and not a waste product, glucose has been invariably conserved by the vertebrates throughout their evolution and at no time continuously rejected from the body. The renal tubules have never been called upon, therefore, to secrete this substance, and are incapable of doing so. There is good evidence that this is equally true of

the mammals. The appearance of glucose in the urine of glomerular animals during hyperglycemia or after phlorizin is in this view an incidental phenomenon referable to incomplete tubular reabsorption of glucose from the glomerular filtrate. But because it is normally reabsorbed by the tubules, glucose itself is not suitable for the evaluation of the glomerular filtrate. Although this reabsorption can be paralyzed by phlorizin there is reason to believe that other physiological activities of the kidney are also influenced by this drug, so that its use is of dubious physiological value.

It seemed that if glucose is not secreted by the tubules, other sugars would not be secreted by them, and that among the metabolically inert sugars one or more might be found which was not reabsorbed. Our attention has been directed toward three which appear to be most suitable: the pentose, xylose; the disaccharide, sucrose; and the trisaccharide, raffinose.

1. *Quantitative determination.* The introduction of the rapid absorption of glucose by washed yeast cells as a method of determining the true glucose content of plasma and urine has made it possible to distinguish this sugar from other reducing sugars, such as xylose. When the concentration of xylose is maintained at a level of 70 mgm. per cent or higher in the plasma, the non-glucose reducing substances of dog plasma and urine can be neglected. (These average about 5 and 150 mgm. per cent, respectively, when using Somogyi's copper precipitation method.) Methods have been devised for the determination of either sucrose or raffinose in the presence of glucose by means of sucrase, for a full description of which the reader is referred to the section of this paper dealing with methods.

2. *Toxicity.* Xylose, sucrose and raffinose can be administered in large amounts without evidence of toxic action. The possibility of a local action upon the kidney will be referred to subsequently in the paper.

3. *The filterable character of the sugars.* There is no evidence that a combination exists in the plasma between glucose and protein, and although it is frequently suggested that the glucose in normal blood exists in some complex condition, there is ample reason to believe that in the phlorizinized animal the glucose is entirely filterable at the glomerulus—a conclusion which is supported by the experiments reported in this paper.

There is no reason to suppose that a physiologically inert sugar such as xylose can combine with protein or otherwise form non-filterable complexes, but to test this point we have filtered dog plasma containing 150 mgm. per cent of xylose through collodion membranes under a pressure of 100 to 150 cm. of Hg; the filtrate contained no protein, as shown by heating and the addition of trichloracetic acid, but the residue and the filtrate contained xylose and glucose in the same concentrations, within the limits of experimental error in the sugar analysis ($\pm$ 2 per cent).

4. *Xylose and sucrose are not secreted by the renal tubules.* We have already referred to Marshall's (1930) observation that the aglomerular

fish kidney is incapable of excreting glucose, even after large doses of phlorizin, indicating that glucose is excreted only by glomerular filtration. Jolliffe (1930) and Clarke and Smith (1932) have shown that xylose and sucrose, although freely excreted by glomerular fish, are not excreted by aglomerular fish from plasma levels of 300 to 400 mgm. per cent.

Though evidence derived from lower vertebrates should not be transferred directly to the higher animals, our rapidly growing knowledge of the comparative physiology of the kidney leads us to believe that sugars are never secreted by the renal tubules. Direct evidence, however, against the secretion of sugars by the renal tubules in the dog is now available in our observations, reported in this paper, that xylose and sucrose, and in the phlorizinized dog xylose and glucose, or raffinose and glucose, are excreted at the same rate relative to the plasma concentration; i.e., the respective *simultaneous glomerular clearances* for these substances are identical. This fact is consonant with filtration, but irreconcilable with secretion.

5. *Xylose is not reabsorbed.* It would appear probable that the normal tubular reabsorption of glucose, which prevails in all vertebrates that have been examined, is related to the metabolic importance of this substance and that the actual process of reabsorption possibly involves to some extent a process of metabolism, a suggestion which has been frequently made. In any case, one would not expect xylose, which is not metabolized to any appreciable extent by the rabbit or the dog (Corley, 1926, 1928; Greenwald, 1931) to be conserved by active tubular reabsorption in the manner of glucose. But if any active reabsorption occurred one would expect phlorizin, because it blocks the reabsorption of glucose, to block the reabsorption of xylose, also.

Xylose is excreted by all normal animals so far examined (glomerular fishes and the dog) with a relatively high U/P ratio when glucose is absent or present only in small quantities, in the urine. This fact in itself indicates that no extensive reabsorption of xylose prevails in the normal animal, but it is not sufficient to warrant the assumption that there is no reabsorption at all, because the quantitative relations between the plasma, the urine and the renal threshold might be different for the two sugars. So we have attempted to answer the question of whether xylose is reabsorbed by comparing the U/P ratios before and after phlorizin of glucose, xylose and a third and indifferent substance—indifferent, that is, so far as sugar metabolism is concerned. In the present experiments on the dog urea has been chosen for this third substance.

Discussion. With the background furnished by the above facts we may turn to the experimental data. The following experiments are given in full:

Table 1. Experiments 87, 88 and 100, showing the excretion of xylose, glucose and urea before and after the administration of phlorizin.

Table 2. Experiments 92 and 96, showing excretion of raffinose, glucose and urea before and after the administration of phlorizin.

TABLE 1

The effect of phlorizin on the excretion of xylose and glucose in the dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		XYLOSE		GLUCOSE		CM. = UV/P/S.A.			CM. GLUCOSE / CM. XYLOSE	CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Glucose		
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*					
87	26	0.885	10.4	271.0	99.1	6,260	121.8	1,090	29.9	72.7	10.3	0.14	0.41
	32	0.906	10.8	225.0	113.5	6,520	125.2	830	24.5	67.6	7.8	0.12	0.36
	31	1.29	11.2	134.5	112.8	3,970	115.8	4,067	20.1	59.0	58.9	1.00	0.34
	28	1.39	11.9	119.0	102.6	3,760	109.0	4,320	18.1	66.2	71.6	1.08	0.27
88	31	0.900			110.0	6,780	85.1	Trace		102.6*	0.0	0.0	
	30	1.066			114.0	5,940	85.0	Trace		102.9	0.0	0.0	
	32	1.312			89.0	3,940	75.7	3,260		107.6	104.6	0.97	
	31	1.290			96.0	4,270	71.2	2,930		106.3	98.4	0.93	
100	24	2.290	6.5	70.7	150.4	2,792			32.8	56.1			0.585
	26	1.537	6.5	98.9	152.7	4,040			30.8	53.6			0.575
	29	1.620	6.5	92.5	169.9	4,185	79.1	1,895	30.1	52.7	51.2	0.972	0.581
Average after phlorizin												0.99	

* Xylose clearance abnormally high, possibly because room was cold and dog shivered throughout experiment.

Expt. 87. Dog 18, wgt. 20 kgm., S.A. 0.77 sq. m. Mixed diet. Twenty-four grams xylose in 60 cc. water injected subcutaneously at 6:00 a.m. and 6 grams in 20 cc. water at 6:45 a.m. Water *ad lib.* Period 1 began at 7:03 a.m.

Two hundred milligrams phlorizin per kgm. in 50 cc. 1.25 per cent $NaHCO_3$ solution injected subcutaneously at end of period 2, or 8:02 a.m. Six grams xylose in 20 cc. water injected subcutaneouly at 8:45 a.m. Period 3 began at 9 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 88. Dog 31, wgt. = 12 kgm., S.A. = 0.54 sq. m. Cracker meal diet. Fourteen and five-tenths grams xylose in 40 cc. water injected subcutaneously at 6:45 a.m. and 3.6 grams in 15 cc. water at 7:30 a.m. Three hundred cubic centimeters water by stomach at 6:50 a.m. and 300 cc. water at 7:30 a.m. Period 1 began at 7:45 a.m.

One hundred milligrams phlorizin per kgm. in 2.5 per cent $NaHCO_3$ injected intravenously and same quantity subcutaneously at end of period 2, or 8:45 a.m. Six grams xylose in 15 cc. water injected subcutaneously at 9:30 a.m. Period 3 began at 9:45 a.m. Blood samples drawn at middle of urine collection periods.

Expt. 100. Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 3 days. Fasted 2 days; 46 grams xylose in 100 cc. water injected subcutaneously at 9:50 a.m. and 11 grams xylose at 10:35 a.m. Period 1 started at 10:56 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ intravenously and same quantity subcutaneously at end of period 2, or 11:55 a.m. Period 3 started at 12:40 a.m. Blood samples drawn at irregular intervals and interpolated.

Table 3. Experiments 84, 85 and 86, showing the excretion of xylose, sucrose and urea in the normal animal.

The facts to be noted in these experiments are as follows:

1. Xylose, sucrose and raffinose are normally excreted by the dog with a relative high U/P ratio, a fact which qualitatively indicates that these sugars are not actively reabsorbed by the tubules.

TABLE 2

The effect of phlorizin on the excretion of raffinose and glucose in the dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		RAFFINOSE		GLUCOSE		CM. = $\frac{UV}{P}$/S.A.			CM. GLUCOSE / CM. RAFFINOSE	CM. UREA / CM. RAFFINOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Glucose		
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*					
92	32	1.940	10.0	197.0	120.6	3,553	87.7	00.0	50.2	75.2	00.0	0.00	0.67
	29	1.380	9.7	262.0	107.4	4,900	84.8	00.0	49.0	82.9	00.0	0.00	0.59
	30	0.933	8.7	303.5	82.0	4,750	79.3	5,010	42.8	71.1	77.6	1.09	0.60
	31	0.967	9.1	276.0	70.9	4,324	78.8	5,075	38.6	77.6	81.9	1.05	0.50
96	30	2.233	6.5	86.4	90.2	2,551	75.5	00.0	39.6	84.4	00.0	0.00	0.47
	33	1.545	6.3	112.3	96.1	3,942	80.3	00.0	36.7	84.5	00.0	0.00	0.43
	49	0.725	6.8	208.3	119.9	5,710	73.7	3,590	29.6	46.0	47.1	1.02	0.64
	34	1.088	6.7	219.9	87.5	5,327	56.5	3,513	47.6	88.4	90.2	1.02	0.54
Average after phlorizin												1.04	

Expt. 92. Dog 20, wgt. 19 kgm., S. A. 0.76 sq.m. Cracker meal diet six weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 300 cc. water by stomach at 6:10 a.m. Six grams raffinose in 30 cc. water subcutaneously at 7:00 a.m. Period 1 began at 7:14 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ solution subcutaneously and same quantity intravenously at end of period 2, or 8:10 a.m. Period 3 began at 9:17 a.m. All blood samples drawn at middle of collection periods.

Expt. 96. Dog 29, wgt. 13.5 kgm., S.A. 0.75 sq. m. Cracker meal diet four weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 500 cc. water by stomach at 9:00 a.m. Six grams raffinose in 30 cc. water subcutaneously at 9:45 a.m. Period 1 began at 8:00 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ solution intravenously and 200 mgm. subcutaneously at end of period 2, or 9:10 a.m. Fourteen grams raffinose in 60 cc. water subcutaneously and 500 cc. water by stomach at 11:40 a.m. Period 3 began at 12:10 p.m. All blood samples drawn at middle of collection periods.

2. Phlorizin does not cause a rise in the absolute value of the U/P ratio for these sugars, as it does in the case of glucose, nor does it cause a rise in clearance (i.e., the quantity of sugar excreted per unit time from a constant blood level, or UV/P) as would be expected if active reabsorption normally occurred and if this reabsorption were blocked by phlorizin.

3. The ratio of the xylose or raffinose clearance to the urea clearance is not significantly affected by phlorizin; there is no reason to believe, even if this drug blocked the reabsorption of these sugars, that it would block the reabsorption of urea to exactly the same extent, if at all; therefore, since the sugar clearance is not increased relative to the urea clearance after

TABLE 3

The excretion of urea, xylose and sucrose in the normal dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		XYLOSE		SUCROSE		$C_M = \frac{UV}{P}/S.A.$			$\frac{C_M \text{ SUCROSE}}{C_M \text{ XYLOSE}}$
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Sucrose	
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*				
84	32	5.72	15.5	110.8	57.1	488	64.5	555	53.8	64.4	64.8	1.01
	31	6.22	15.3	108.0	78.3	571	94.6	688	57.8	59.6	59.6	1.00
	33	4.27	15.2	145.5	69.0	816	75.0	915	53.8	66.5	68.6	1.03
85	32	1.08	12.5	165.3	98.1	1,652	109.2	1,880	26.4	33.7	34.4	1.02
	28	1.09	12.9	146.5	106.4	2,019	102.4	2,116	22.9	38.3	41.7	1.09
	30	1.03	13.5	175.0	116.4	2,086	115.2	2,308	24.7	34.2	38.2	1.12
86	31	1.03	10.5	257.0	95.8	4,025	104.8	4,075	33.2	57.0	52.7	0.92
	29	1.17	11.0	215.0	105.2	4,130	108.8	4,398	30.1	60.6	62.2	1.03
	32	1.22	9.5	203.0	101.3	3,820	109.1	4,210	34.3	60.6	62.0	1.02
Average												1.01

Expt. 84. Dog 20, wgt. 19.4 kgm., S.A. 0.76 sq. m. Cracker meal diet 11 days. Fifteen grams each xylose and sucrose in 60 cc. water injected subcutaneously at 6:00 a.m., and again at 8:00 a.m. Seven hundred fifty cubic centimeters water by stomach at 5:55 a.m. and 500 cc. at 7:55 a.m. Period 1 began at 8:08 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 85. Dog 31, wgt. 12 kgm., S.A. 0.54 sq. m. Cracker meal diet 22 days. Nine grams each xylose and sucrose in 36 cc. water injected subcutaneously at 6:15 a.m. and again at 7:00 a.m. Four hundred cubic centimeters water by stomach at 6:20 a.m. and 200 cc. at 7:05 a.m. Period 1 began at 7:15 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 86. Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 31 days. Thirty-two grams xylose and 24 grams in 100 cc. water injected subcutaneously at 6:05 a.m. and 8 gms. xylose and 6 grams sucrose in 20 cc. water at 6:50 a.m. No water given. Period 1 began at 7:06 a.m. All blood samples drawn at middle of urine collection periods.

phlorizin, it may be inferred that phlorizin is without effect upon the former.

4. Under the influence of phlorizin the glucose clearance rises to the xylose clearance or the raffinose clearance, as the case may be, but never significantly exceeds the latter. This indicates that there is no active reabsorption of xylose or raffinose which is *not affected* by phlorizin; for if

such reabsorption existed then the glucose clearance under phlorizin should rise above the xylose or raffinose clearance. This conclusion would be invalid if the action of phlorizin in blocking the reabsorption of glucose were an incomplete one, but all the evidence available on this subject indicates that in sufficient doses the contrary is the case; in fact, it has been observed in this laboratory that as little as 25 mgm. of phlorizin per kilogram given intravenously suffices to bring the glucose clearance up to that of xylose for short periods of time (1 to 2 hours), and larger doses do not raise it any higher. In experiments 88, 92, 96 and 100, we have given four times this quantity intravenously and an equal quantity subcutaneously, so we believe that the reabsorption of glucose is completely blocked.

We conclude from the above facts that there is no active reabsorption of xylose or raffinose in the kidney of the normal dog.[1]

It remains possible, however, that a small quantity of sugar might be reabsorbed into the blood stream by passive diffusion from the lumen of the tubule, since during the reabsorption of water a large concentration gradient is established between the urine and the plasma. That such a passive diffusion must be slight is indicated by the fact that urea can be concentrated 250 times, and urea is more diffusible than any of the sugars; but since it is possible that urea is itself extensively reabsorbed, this evidence is hardly conclusive. Therefore, to examine this question further we have compared the excretion of xylose with sucrose in the normal dog. Sucrose is not excreted by the tubules of *Lophius*, as shown by Clarke and Smith (1932), in any detectable quantity; certainly less than one per cent diffuses across the renal tubules from the plasma into the urine. (The excretion of raffinose by the aglomerular kidney has not been examined.) The molecular weights of unhydrated xylose, sucrose and raffinose are 150, 342 and 504, respectively, and it is to be expected that their rates of diffusion would vary inversely as the molecular size.

5. The data given in table 3 show that xylose and sucrose are excreted by the normal kidney in an identical manner within the limits of the ex-

[1] It seems hardly necessary to discuss the view that phlorizin induces the secretion of glucose by the renal tubules. The extensive studies which have been made on the *modus operandi* of this drug have demonstrated to the satisfaction of most investigators that it acts by paralyzing the tubular reabsorption of glucose from the glomerular filtrate (Nash, 1927). There is conclusive evidence in Marshall's (1930) work on the fishes that the renal tubules are unable to secrete glucose and this fact, even if there were no other evidence, would lead us to believe that the tubular secretion of glucose in the mammals is a fiction. But we now have the additional evidence in our subjoined tables to the effect that the physiologically inert sugars, xylose, sucrose and raffinose, when excreted simultaneously are excreted in an identical manner (i.e., with an identical clearance) in the normal animal; a principle which is extended to glucose in the phlorizinized animal. These observations leave no doubt whatsoever that the excretion of all these sugars is effected exclusively by glomerular filtration.

perimental error in the determination of the sugars. The data given in tables 1 and 2 show that under phlorizin, glucose and xylose, on the one hand, and glucose and raffinose, on the other, are likewise excreted in an identical manner; so that except for the use of phlorizin to block the reabsorption of glucose, we may say that all these sugars are handled simultaneously by the kidney in exactly the same way. It would seem, therefore, that they must be reabsorbed to exactly the same extent, or else not reabsorbed at all. Since we are comparing substances of different molecular weights and diffusibility, the first alternative is very unlikely. This conclusion is reinforced by the observations of Clarke and Smith (1932) that the tubules of *Lophius* do not permit xylose and sucrose to diffuse across them, whereas urea is nearly uniformly distributed between the blood and urine (Marshall and Grafflin, 1928). In the dog, on the other hand, where urea may be concentrated 250-fold it is inconceivable that the tubules would permit the passive diffusion of appreciable quantities of substances like raffinose, sucrose, xylose and glucose.

In résumé, the facts that the xylose clearance relative to the urea clearance is unaffected by phlorizin, that the glucose clearance under phlorizin does not exceed the xylose clearance or the raffinose clearance, and that the xylose and the sucrose clearances in the normal animal and the glucose and xylose or glucose and raffinose clearances in the phlorizinized animal are identical (in simultaneous experiments), are interpreted to indicate that neither xylose, sucrose nor raffinose is reabsorbed by the renal tubules, and therefore, in vew of the arguments set forth above, any of these substances may be used to measure the glomerular filtrate with a theoretical accuracy lying within the limits of the experimental error in the sugar analysis.

Of the three sugars examined xylose and sucrose are the most suitable for practical purposes. The accurate determination of xylose is perhaps not so easy as is the determination of sucrose, but the former possesses the advantages that it can be administered *per os* and its chemical determination is not dependent upon the use of an enzyme.

The question of whether the administration of these sugars influences the excretory activity of the kidney with respect to other substances cannot be answered in this paper. It would seem that an inert sugar would exert as little action as any substance which might be used for the measurement of the glomerular clearance. But it is recognized that many factors, only a few of which are known at present, modify the activity of the kidney, and although we believe that these sugars have no specific stimulating or depressing action, either in respect to the excretion of urea or any other substance, the examination of this point must be postponed until certain conditions bearing on the maintenance of uniform renal activity can be discussed.

SUMMARY

It is shown that xylose is excreted by the normal dog kidney, at moderate to large urine flows, with a relatively high U/P ratio; under the action of phlorizin the glucose clearance rises to but never exceeds the xylose clearance, while the xylose clearance itself, or the xylose clearance relative to the urea clearance, remains unaffected. These facts indicate that there is no active reabsorption of xylose by the renal tubules.

The pentose, xylose, the disaccharide, sucrose, and the trisaccharide, raffinose, all of which are physiologically inert, are excreted (in simultaneous experiments) in an identical quantitative manner relative to each other or to glucose under phlorizin, indicating that there is no significant passive reabsorption (by diffusion) from the concentrated urine in the tubules.

From these and other considerations described in the paper we believe that these sugars can be used to evaluate the quantity of glomerular filtrate with an error that does not exceed a few per cent.

Methods for the analysis of mixtures of xylose, glucose and sucrose, or of glucose and raffinose in plasma and urine are described.

The xylose used in these experiments was kindly supplied by the Swann Chemical Company of Birmingham, Alabama. We are indebted to Miss Annie Breitweiser for her expert technical services in sugar analysis.

METHODS. A careful comparison of several sugar methods, tested by the recovery of added amounts of various sugars to plasma and urine, led us to choose the Folin (1929) method as yielding the best results in our hands. The Folin sugar reagent possesses the advantage that it is not reduced by phlorizin, which substance reduces ferricyanide; this fact, in our opinion, renders suspect all quantitative glucose observations on phlorizinized animals made with a ferricyanide method. The preparation of reagents and the sugar analysis were carried out precisely as described by Folin except that we have used copper filtrates as prepared by Somogyi (1931). Special blow-out pipettes were used in all measurements and great care was exercised in all details of technique.

Glucose + xylose. A portion of serum or oxalated plasma is added to 7 volumes of water in a 125 cc. Erlenmeyer flask; one volume of copper solution and one volume of sodium tungstate solution are added while spinning and the precipitated proteins are filtered out after 15 minutes using S. and S. 597 filter paper. Two cubic centimeters of this filtrate are taken for sugar analysis according to Folin (1929). Urine is diluted according to the expected sugar content, usually 1:25 or 1:50, with 0.06 per cent benzoic acid. A copper filtrate is prepared from this diluted urine as above. Glucose, xylose and sucrose will remain unchanged in the diluted urine over-night if kept in the ice-box.

Xylose. A weighed sample of yeast is made up to a 20 per cent suspension in water and washed by centrifuging until the supernatant fluid is clear (usually four times); 5 cc. of this suspension are centrifuged in a 15 cc. centrifuge tube for 15 minutes at high speed, decanted and drained for 30 minutes, when the sides of the tube are wiped dry up to the edge of the yeast. Five cubic centimeters of plasma or urine filtrate are added to the 1 cc. of dry yeast, stirred well and allowed to stand at room

temperature for 15 minutes with occasional agitation. The mixture is then centrifuged for 15 minutes to throw down the yeast and 2 cc. of the supernatant fluid are used for sugar analysis. (After Somogyi, 1928, and Van Slyke and Hawkins, 1929.)

We find that xylose is not actively absorbed by yeast, but that a small quantity (depending upon the proportion of yeast and filtrate) disappears each time during successive yeast treatments. This fraction is about 13 per cent when the proportions of 1 cc. of dry yeast to 5 cc. of fluid are used. This absorption probably represents a moiety which diffuses into the yeast. (Cf. also Van Slyke and Hawkins, 1929, who found partial absorption of non-fermentable substances.) In this respect we do not confirm Raymond and Blanco (1928) who obtained complete recovery of xylose after yeast treatment and who make no mention of passive diffusion. The reducing power of pure xylose by the Folin sugar method in our hands is 85 per cent of that of glucose in contrast to 78 per cent obtained by Poe and Klemme (1930); consequently after yeast treatment as above we recover 72 per cent of added xylose. This low recovery is theoretically and actually constant and can be neglected in the calculation of U/P ratios when the plasma and urine filtrates are treated in exactly the same manner. Consequently we do not make any correction in our data for reducing power of xylose, but present only the observed glucose equivalent.

In calculating the true glucose, however, allowance must be made for the diffusion of xylose into the yeast; therefore we calculate the glucose content of both plasma and urine as glucose + apparent xylose—apparent xylose/0.87.

Sucrose. The total sugar content of plasma and urine filtrates prepared as above, and containing glucose, xylose and sucrose was determined by inversion with sucrase. We are indebted to Mr. Milton Levy, of the Department of Biochemistry, for the sucrase used in these experiments. The enzyme was prepared from yeast by Hudson's method (cf. Morrow, 1927); we use a 0.26 per cent solution of a preparation 50 mgm. of which reduces the rotation of 20 cc. of 20 per cent sucrose solution to zero degrees in twelve minutes.

We find that sucrose is quantitatively absorbed by yeast, either the small tin-foil packages or baker's loaves, almost instantly. One cubic centimeter of yeast completely removes 40 mgm. from 5 cc. of solution (corresponding to 800 mgm. per cent in our method) in less than 5 minutes. In this observation we do not confirm Raymond and Blanco (1928) who observed only partial absorption, possibly because the yeast which they used was not fresh. (Cf. also Ronzoni and Somogyi, 1929.)

One cubic centimeter of plasma or urine filtrate is placed in a Folin sugar tube with one drop of 0.04 per cent bromocresol green, and 0.05 N acetic acid is added drop by drop until the color indicates a pH between 4.0 and 4.6. One cubic centimeter of sucrase is added and the mixture is agitated and allowed to stand in a water bath at 40°C. for 30 minutes or more after which it is neutralized with 0.05 N NaOH. One drop of phenolphthalein is added and the mixture is made alkaline with one per cent Na_2CO_3 as in Folin's sugar method. The standards are treated by adding the bromocresol green, acetic acid and NaOH. 2.0 cc. of Folin reagent are then added and the routine sugar analysis completed. Blanks must be made on the sucrase solution by adding it to known glucose solutions.

Sucrose is calculated as the difference between the glucose equivalents before and after inversion. The glucose equivalent of pure sucrose solutions treated as above is 105 per cent of glucose, and we obtain recoveries of added amounts (100 to 300 mgm. per cent) from plasma and urine with an error of $\pm$ 2 per cent. In the Folin method the reducing power of glucose is not impaired by the presence of sucrose in approximately equal concentrations, and since sucrose is completely removed by yeast (cf. above) it offers no interference to the determination of xylose.

Raffinose. Raffinose (a non-reducing trisaccharide) is hydrolyzed by sucrase to fructose and melibiose. Both sugars reduce Folin's Cu reagent, but the latter is

not absorbed on yeast. By the above method we find the reducing power of hydrolyzed raffinose at concentrations of 100 to 200 mgm. per cent to be 60 per cent of glucose. Since melibiose is not absorbed by yeast, it is not possible to determine xylose in the presence of raffinose by sucrase hydrolysis, so we have compared the excretion of raffinose with glucose under phlorizin, the analyses being made in the same manner as in the case of sucrose. Our data express glucose equivalents of raffinose as calculated from the difference between the glucose equivalents of solutions before and after hydrolysis.

When present in approximately equal concentrations raffinose does not interfere with the quantitative determination of glucose.

All plasma and urine filtrates are prepared in duplicate and these duplicates are analyzed separately with separate standards. All analyses in which the unknown differs from the standard by more than 20 per cent are repeated, using a more appropriate standard. No standard corresponding to more than 200 mgm. per cent or less than 50 mgm. per cent was used.

Urea. Urea in both plasma and urine was analyzed by Van Slyke's (1927) plasma method, the plasma and urine analyses being alternated to prevent poisoning of the urease in the extraction chamber. One or 2 cc. samples of plasma and 1 cc. of diluted urine were used. All analyses were run in duplicate for 3 and 6 minutes, the variation in time serving to check the activity of the urease. The agreement between checks, even at concentrations of 6 mgm. per cent is usually better than 2 per cent.

General. Except as noted the general technique is similar to that used by Jolliffe and Smith (1931a, b). The dogs were maintained upon a cracker-meal and butter or lard diet. The greatest care was exercised in catheterizing to insure complete removal of urine from the bladder. In the present experiments the sugars were injected subcutaneously some time before the first urine collection period; injection is not necessary in the case of xylose which may be given by stomach, but sucrose and raffinose must obviously be administered parenterally. The essential details of each experiment are recorded in the tables.

Note on the filterable nature of plasma glucose (*cf. p. 302*): After submitting this paper we noted the work of Powers and Greene (1931) showing that all the plasma glucose is filterable by *in vivo* dialysis.

BIBLIOGRAPHY

Behre, J. A. and S. A. Benedict. 1922. Journ. Biol. Chem., lii, 11.

Clarke, R. W. and H. W. Smith. Journ. Cell. Comp. Physiol., (in press).

Corley, R. C. 1926. Journ. Biol. Chem., lxx, 521.
1928. Journ. Biol. Chem., lxxvi, 23.

Folin, O. 1929. Journ. Biol. Chem., lxxxii, 83.

Gaebler, O. H. 1930. Journ. Biol. Chem., lxxxi, 451.

Gaebler, O. H. and A. K. Keltch. 1928. Journ. Biol. Chem., lxxvi, 337.

Greenwald, I. 1931. Journ. Biol. Chem., xci, 731.

Jolliffe, N. 1930. Proc. Soc. Exp. Biol. Med., v, xxviii.

Jolliffe, N. and H. W. Smith. 1931a. This Journal, xcviii, 572.
1931b. This Journal, xcix, 101.

Marshall, E. K., Jr. 1930. This Journal, xciv, 1.

Marshall, E. K., Jr. and A. L. Grafflin. 1928. Bull. Johns Hopkins Hospital, xliii, 205.
1932. Journ. Cell. Comp. Physiol., (in press).

Marrow, C. A. 1927. Biochemical laboratory methods for students of the biological sciences. New York.

Nash, T. P., Jr. 1927. Physiol. Rev., vii. 385.

POE, C. F. AND KLEMME, D. 1930. Journ. Biol. Chem., lxxxvii, 7.
POWERS, M. H. AND C. H. GREENE. 1931. Journ. Biol. Chem., xciv, 281.
RAYMOND, A. L. AND J. G. BLANCO. 1928. Journ. Biol. Chem., lxxix, 649.
REHBERG, P. B. 1926. Biochem. Journ., xx, 447.
RONZONI, E. AND M. SOMOGYI. 1929. This Journal, xc, 499.
SOMOGYI, M. 1928. Journ. Biol. Chem., lxxviii, 117.
1931. Journ. Biol. Chem., xc, 725.
VAN SLYKE, D. D. 1927. Journ. Biol. Chem., lxxiii, 695.
VAN SLYKE, D. D. AND J. A. HAWKINS. 1929. Journ. Biol. Chem., lxxxiii, 51.

ACTION OF EPINEPHRINE ON THE INTESTINE FOLLOWING STIMULATION BY PARASYMPATHETIC DRUGS

FREDERICK BERNHEIM AND BERGET H. BLOCKSOM, JR.

From the Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.

Received for publication January 14, 1932

There have been occasional and sporadic reports in the literature of an anomalous action of epinephrine on the intestine; namely, the addition of this drug causes a contraction. Thus Salvioli (1902) injected epinephrine intravenously into a cat and observed after a small relaxation a marked contraction of the ileum. This was confirmed by Magnus (1903). In corroboration with this Bunch (1898) showed that electrical stimulation of the splanchnics may cause intestinal contractions. All these experiments were done with the intestine in situ and the nerve supply intact. One observation reported by Sharpey-Schäfer (1924) in his book shows an isolated piece of intestine contracted by the addition of epinephrine. Recently these observations have been confirmed in the case of ephedrine by Kreitmair (1927) and Kinnaman and Plant (1931) who show that large concentrations of ephedrine contract the intestine. They assume that ephedrine acts on the intrinsic motor mechanism (Auerbach's plexus) of the intestine, because in plexus free preparations no contraction occurs. Magnus observed this also in the case of epinephrine.

While working with isolated intestinal strips, we observed that the addition of epinephrine to intestine already partially contracted by pilocarpine always caused a greater contraction followed after some time by a slow relaxation. The rapidity with which relaxation set in varied with different preparations. Occasionally it immediately followed the rise, in other cases the rise was maintained for as long as a minute, and in still other cases no appreciable relaxation occurred. The rise, however, was a constant factor, the height of which depended on how far the intestine was already contracted by the pilocarpine. These results were also obtained when pilocarpine was substituted by physostigmine, or under certain conditions by histamine. In no case did we observe a contraction when epinephrine was added alone.

The interaction of pilocarpine and epinephrine was first worked out by Hirz (1913) who showed that these drugs worked antagonistically on the intestine of dogs and cats. Although his curves show no rise after the

addition of epinephrine, there is a distinct latent period before relaxation sets in. Recently Takeda (1930) has shown that epinephrine acts most powerfully as depressant on unstimulated rabbit's intestine, but after stimulation with pilocarpine or physostigmine the relaxation is almost abolished.

EXPERIMENTAL. The Burn and Dale apparatus was used. The experiments were performed on the intestine of guinea pigs which were starved 24 hours and lightly anesthetized with ether before decapitation and removal of the ileum. Pieces 2 cm. long were tied off with an empty lumen and suspended in the bath at 37°C. A modified Ringer solution without phosphate carbonate or glucose was used aerated with carbon dioxide free air. The intestine was suspended in 45 cc. of this solution.

Fig. 1. First curve: contraction with 0.2 cc. pilocarpine followed by 0.2 cc. epinephrine added at arrow. Second curve: Contraction with 0.2 cc. histamine, 0.2 cc. epinephrine added at arrow. Third curve: The first contraction due to 0.01 cc. nicotine, the second contraction to 0.2 cc. histamine. 0.2 cc. epinephrine added at arrow. The three curves obtained on the same piece of ileum. The different height of contraction is not a factor. Similar results are obtained when the response to histamine and pilocarpine is the same.

The drugs were made up in the following concentrations: pilocarpine sulfate (Merck) 5 mgm. in 25 cc.; physostigmine hydrochloride (Merck) 10 mgm. in 25 cc.; histamine acid phosphate (British Drug Houses) 2.5 mgm. in 25 cc.; and adrenalin (Parke, Davis) 1 part in 1000. Both the adrenalin tablets and the solution containing chloretone were used with similar results.

The first curve in figure 1 shows the effect of 0.2 cc. of epinephrine added after contraction by 0.2 cc. of pilocarpine. In this curve the intestine shows no peristalsis, but in preparations where there is active peristalsis the same phenomenon occurs. If physostigmine is substituted for pilocarpine, epinephrine has the same effect. Variations in the amount of pilocarpine added up to 1 cc. did not affect the result. Nor did the concentration of epinephrine even when as much as 2 cc. were added

affect the shape of the curve. If the intestine was contracted to its fullest extent, the rise after epinephrine would be very slight and occasionally absent. To avoid this only small amounts of pilocarpine were added.

When histamine is substituted for pilocarpine, the addition of epinephrine very seldom causes a contraction, but there is a distinct latent period before the relaxation occurs. Should a contraction occur, it is always immediately followed by a relaxation and the contraction is inconspicuous compared to the one caused by epinephrine in the presence of pilocarpine. Histamine acts primarily directly on smooth muscle but it also has some action on the parasympathetics, for atropine will cause a relaxation after histamine as shown by McKaye (1930) and Bernheim (1931). Nicotine will inhibit the contraction by histamine to about the same extent as atropine. In the presence of nicotine or atropine the latent period seen on the addition of epinephrine after histamine alone is considerably shortened and occasionally completely abolished and the extent of the relaxation is much greater. This is shown in curves 2 and 3 in figure 1. The atropine curve is similar to the nicotine curve shown here. The three curves in figure 1 were obtained on the same strip of intestine which was thoroughly washed between the additions of the drugs. When the nicotine is washed away, a curve similar to the second curve is obtained.

The duodenum behaves similarly to the ileum in all these respects, but the action of epinephrine is not as clear cut or as regular as on the ileum.

Discussion. It is obvious from these experiments that parasympathetic stimulation is necessary before a contraction by epinephrine is possible. Thus after pilocarpine or physostigmine contractions by epinephrine are regularly seen whereas after histamine contractions are rarely seen, but there is a definite latent period before relaxation. This corresponds to the fact that histamine stimulates the parasympathetic very slightly compared to pilocarpine and physostigmine; moreover, parasympathetic poison such as atropine and nicotine inhibit to a certain extent the histamine contraction, cut down the latent period, and increase the relaxation by epinephrine. As regards the older observations on the contraction of the intestine by epinephrine, it is significant that with the exception of a single observation by Sharpey-Schäfer, they were made on the intestine in situ with the nerves intact, where presumably vagus tone if not actual stimulation was in effect.

Epinephrine, a sympathetic stimulant, acts further to contract guinea pig's intestine already partially contracted by parasympathetic stimulation. This fact, with the observation of Carlson, Smith and Gibbons (1927) that acetylcholine may contract or relax cat's intestine depending upon its state, the observation of Bernheim (1931) that pilocarpine may relax a contracted duodenum of the guinea pig, plus the additional evidence that electrical stimulation of the vagus may cause intestinal relaxation

in the cat (Melik-Megrabow, 1926), in the turtle (Rogers and Bercovitz, 1921) in the dog (Bercovitz and Rogers, 1921) and that electrical stimulation of the splanchnics may cause intestinal contraction in the dog (Bunch), indicates that the conception of a rigid antagonism between the sympathetics and parasympathetics in the intestine is no longer strictly valid. Instead the reaction of one of these systems is not immutable, but is a function of the reactivity of the other or the state of the intestine itself. Thus under one set of conditions they may be antagonistic, under another they may reinforce one another, and under a third their usual rôles may be reversed.

It might be well to add here that, due to the difference in the action of epinephrine after pilocarpine and after histamine, the contraction caused after the former cannot be due to impurities in the commercial preparation but must be due to the epinephrine itself. Also we have occasionally had intestines which reacted very slowly to pilocarpine. In these cases addition of epinephrine causes a contraction much greater than those recorded above but because the pilocarpine action was not typical we have not included a record of them here.

SUMMARY

1. Epinephrine added to the ileum of a guinea pig which has been contracted by pilocarpine or physostigmine causes a further contraction which may or may not be followed by relaxation.

2. After histamine, epinephrine always causes relaxation, but the relaxation occurs after a definite latent period. The relaxation is more rapid and the latent period shorter in the presence of nicotine or atropine.

3. These facts indicate that epinephrine may act as an adjuvant to parasympathetic drugs and that its action is dependent on the amount of parasympathetic stimulation.

BIBLIOGRAPHY

Bercovitz, H. and F. T. Rogers. 1921. This Journal, lv, 323.
Bernheim, F. 1931. Journ. Pharm. Exper. Therap., xliii, 509.
1931. Journ. Pharm. Exper. Therap., xlii, 441.
Bunch, J. L. 1898. Journ. Physiol., xxii, 357.
Carlson, A. J., E. A. Smith and I. Gibbons. 1927. This Journal, lxxxi, 431.
Hirz, O. 1913. Arch. f. Exp. Path. u. Pharm., lxxiv, 319.
Kinnaman, J. H. and O. H. Plant. 1931. Journ. Pharm. Exper. Therap., lxiii, 477.
Kreitmair, H. 1927. Arch. f. Exp. Path. u. Pharm., cxx, 189.
Magnus, R. 1903. Ergebn. d. Physiol., ii, 637.
McKaye, M. E. 1930. This Journal, xc, 527.
Melik-Megrabow, A. M. 1926. Pflüger's Arch., cxiii, 245.
Rogers, F. T. and H. Bercovitz. 1921. This Journal, lvi, 257.
Salvioli, T. 1902. Arch. Ital. d'Biol., xxxvii, 386.
Sharpey-Schäfer, E. A. 1924. The endocrine organs. 2nd ed., p. 133.
Takeda, M. 1930. Folio Pharm. Japan, x, 100.

STUDIES OF GALL-BLADDER FUNCTION

VII. The Anion-Cation Content of Hepatic and Gall-Bladder Bile[1]

I. S. RAVDIN, C. G. JOHNSTON,[2] C. RIEGEL and S. L. WRIGHT, Jr.[3]

From the Laboratory of Research Surgery and the John Herr Musser Department of Research Medicine, University of Pennsylvania

Received for publication January 21, 1932

We have previously reported the fate of various concentrations of sodium chloride, calcium lactate, bile salts, cholesterol and a mixture of bile salts and cholesterol when placed separately in the bile-free gall bladder of the unanesthetized dog (1932a, b, c). The method used in those studies permitted us to study fluids quantitatively in a gall bladder which was bile-free, sterile and at the same time retained its blood supply and major lymphatic vessels. Furthermore, hyperbilirubinemia did not occur and the feces were always normal in color.

It seemed highly desirable to use the same method in order to study the changes in the anion-cation concentration of hepatic bile when this was subjected to gall-bladder activity. We are not aware of any studies of anion-cation balance of hepatic and gall-bladder bile. Gamble and McIver (1928) published data on the total base and chloride of the hepatic and gall-bladder bile of the cat. They did not study the bicarbonate or bile acid concentration, nor were their studies quantitative in that they did not subject known amounts of hepatic bile, or measured concentrations, to gall-bladder activity. From the studies which they made definite conclusions cannot be drawn as to whether the observed differences in the bile were due to absorption, to secretion or excretion, or to a combination of these processes.

Method. The hepatic bile was obtained from cholecystectomized dogs, following double catheterization of the common duct. By this method we were able to collect bile at will or to shunt it into the distal portion of the duodenum. The proximal catheter was inserted to a point just below the insertion of the right and left lobe ducts. The distal catheter was introduced to the point where the common duct began to traverse the

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] Harriet M. Frazier Fellow in Surgical Research.

[3] Robert Robinson Porter Fellow in Research Medicine.

duodenal wall. If the catheter was inserted into the duodenal lumen infection was much more apt to occur.

The bile was collected for twenty-four hours and then the flow was turned into the distal catheter. Thus the animals were kept healthy and the appetite did not suffer materially. The operations as well as all collections were done under strictly aseptic precautions. Cultures or smears were made frequently and the animals were discarded when evidence of infection was obtained.

The dogs used for the study of gall-bladder activity were prepared by the same method which we used in our previous studies (1932a). The same care was taken in these animals as was taken with the fistula animals to prevent infection.

Only data from animals whose gall bladders were proven to be sterile are being reported in this paper. The activity of the damaged gall bladder is so different from the normal that we are reporting the observations on the damaged gall bladder separately.

The gall bladder of every animal used for the activity experiments was first filled with a solution of sodium chloride (152 m.Eq. per liter) after carefully cleansing it with this solution. Every preparation was tested at operation so as to be sure that we could recover the exact amount of fluid introduced. Animals with unligated accessory ducts were discarded, as were animals where the rate of absorption of the sodium chloride was not comparable to that which we had observed in the earlier experiments (1932a). The experiments were begun after recovery from the anesthetic.

Hepatic bile in known amounts was introduced into the bile-free gall bladder and permitted to remain there for from two to twenty-four hours. It was removed with a Luer syringe. As long as the gall bladder remained normal fluid was absorbed. The exact amount varied considerably, the maximum being 6.75 cc. per hour, the rate decreasing as the bile became concentrated. Estimations of the pigment concentrations made by us in hepatic bile subjected to gall-bladder activity have shown increases in concentration of this substance over a 24 hour period varying from 5 to 17 times that of the bile introduced.

The methods used in our analyses were substantially the same as those reported in our earlier papers (1932a, b, c).

The pH determinations were done with the glass electrode of Stadie, O'Brien and Laug (1931).

The freezing point determinations were done by Sunderman using the method of Stadie and Sunderman (1931).

RESULTS. *Hepatic bile.* This bile is more variable in its electrolyte composition than is serum. This is true for hepatic bile from different animals and for the same animal at different times. The variation occurs chiefly in the ratio between chloride, bile salt and bicarbonate (table 1).

We have made no attempt to study the total 24 hour anion-cation output so that we cannot state whether taken for such a period the anion-cation content of hepatic bile in the same animal is constant under similar conditions. The total base concentrations of hepatic bile have varied from 174 to 192 m.Eq. per liter.

On the whole, hepatic bile is higher in base, total anion and as a rule in bicarbonate than is serum. The chloride concentration of hepatic bile tends to be somewhat lower than that of serum. The bile salt concentration has varied from 15.6 to 66 m.Eq. per liter. There is considerable variation in the calcium concentration of the hepatic bile at different periods in the same dog and in different dogs. We have obtained concentrations varying from 3.1 to 9.9 m. Eq. per liter. In the majority of instances the concentration is higher than that of serum. The pH has varied from 7.1 to 8.6. As a rule it is above that of serum.

TABLE 1

Hepatic bile data

DOG NO.	BASE	CALCIUM	CHLORIDE	CO_2	BILE SALT
	m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
535		3.4	55.0	64.8	42.0
96	181	6.3	103.9	34.4	29.6
96	179	5.8	89.6	45.1	28.1
96	183	6.5	63.7	62.8	30.0
91	188	5.9	62.1	58.9	42.1
91	192	9.9	93.2	34.7	45.9
188	189	6.9	89.2	45.1	32.9
188	174	7.0	93.6	40.6	36.7
188	176	6.9	107.0	45.8	34.2

The amount of anion which might be accounted for by the small amount of protein present in hepatic bile of the dog is not more than a few milli-equivalents per liter. A comparison of the determined anion-cation concentrations shows that unknown anions account for a median of about 12 milli-equivalents per liter. The amount of cation bound by phosphoric acid either inorganic or in lipoid combination is unknown. No attempt was made to determine the concentration of fatty acids or ethereal sulphates in hepatic bile.

Gall-bladder bile. After subjecting a single injection of hepatic bile to gall-bladder activity for a short period (average 5 hours) there was considerable variability in the composition of the bile. In seven of the nine short experiments where complete studies were made the per cent decrease of total determined anion was greater than that of cation. In the remaining two experiments total determined anion showed the same per cent decrease as cation.

There is apparently no correlation between the exact time intervals and the changes in the anion concentration which occurred during the short period. The undetermined anion concentration increased in the gall-bladder bile to a median of 26 m.Eq. per liter. Whatever this may be we

TABLE 2

Anion-cation studies

Time average 5 hours

DOG NO.	HEPATIC BILE						GALL-BLADDER BILE					
	Total base	Measured anion	Chloride	Bile salt	$BHCO_3$	pH	Total base	Measured anion	Chloride	Bile salt	$BHCO_3$	pH
	m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*		*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	
122 (1)	184.0		94.3		22.2	7.9	173.0		70.3		14.8	6.7
(3)	177.0		94.5	24.1			167.0		50.4	43.0		
184 (2)	181.0	167.9	103.9	29.6	34.4	7.2	178.0	162.4	91.6	65.2	5.6	6.3
(3)	179.0	162.8	89.6	28.1	45.1	7.1	157.0	142.1	102.0	34.4	5.7	6.4
(4)	177.0	173.1	84.5	61.0	27.6	7.5	154.0	134.8	99.2	31.4	4.2	6.5
(5)	183.0	156.5	63.7	32.0	62.8	8.1	169.0	132.0	77.2	24.3	30.5	7.0
(7)	188.0	163.1	62.1	42.1	58.9	7.4	178.0	152.5	86.8	45.7	20.0	6.7
190 (1)	192.0	173.8	93.2	45.9	34.7	7.9	210.0	169.5	70.6	77.8	21.1	7.2
(3)	189.0	167.2	89.2	32.9	45.1	7.9	198.0	133.5	27.6	61.6	44.3	7.5
(4)	174.0	170.9	93.6	36.7	40.6	7.8	167.0	139.7	47.8	34.4	57.5	7.4
(5)	176.0	187.0	107.0	34.2	45.8	8.2	151.0	141.6	70.2	37.8	33.6	7.4

TABLE 3

Summary of data of short experiments

	NUMBER OF EXPERIMENTS	INCREASE IN CONCENTRATION	DECREASE IN CONCENTRATION	HEPATIC BILE		GALL-BLADDER BILE	
				Maximum	Minimum	Maximum	Minimum
				m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
Base	11	2	9	192.0	174.0	210.0	151.0
Calcium	20	20	0	9.7	5.3	25.7	6.9
pH	17	0	17	8.6	7.1	7.5	6.3
$H\bar{C}O_3$	15	1	14	62.8	22.2	57.5	3.2
Free CO_2	10	9	1	4.5	0.4	5.9	1.1
Chloride	31	5	26	107.0	62.1	102.0	1.9
Bile salt	27	22	5	65.7	15.6	194.2	24.3
X anion	9	8	1	26.9	0.0	62.4	7.7

have called it the "x" anion. It increases in concentration when hepatic bile was subjected to gall-bladder activity.

In table 2 are included sufficient data to show the types of variation which we have observed in these short experiments and in table 3 a sum-

mary of all the short experiments. In the latter complete data are at times lacking. Total base as a rule decreased in concentration while the calcium concentration increased consistently, and bile salt concentration increased in 82 per cent of the experiments. Bicarbonate concentration decreased, and in 84 per cent of the experiments the same was true for chloride. The free CO_2 increased and the pH of hepatic bile decreased when subjected to gall-bladder activity.

In the two experiments where total base concentration increased the sum of the bile salt and undetermined anion concentrations increased to above

TABLE 4

Hepatic bile subjected to gall-bladder activity—additive experiments

DOG NO.	DATE		HEPATIC BILE INTRODUCED	RECOVERED FROM GALL BLADDER	REMOVED FOR ANALYSIS	TOTAL BASE	CALCIUM	CHLORIDE	CO_2	BILE SALT
			cc.	*cc.*	*cc.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
453	11/13/31	Bile removed*				284	25.6	4.1		239
	11/13/31		28.0	2.5	0.0					
	11/14/31		31.0		4.0		26.3	5.6		114
	11/15/31		31.0	5.0	5.0	224		7.3		227
410	11/17/31	Bile removed*					23.4	10.3		189
	11/17/31		20.0	1.8	0.0					
	11/18/31		37.0	7.0	7.0	241	26.4	8.2		282
490	11/23/31	Bile removed*				305	30.9	1.1	0.9	334
	11/23/31		44.0	3.5	0.0					
	11/23/31		17.0							
	11/24/31		24.0	4.5†	0.0					
	11/24/31		7.0		7.0‡	235		33.7	16.9	229
	11/24/31				7.0	240	31.1	2.1	6.7	252

* Gall-bladder bile removed at time of operation.

† Recovered before addition of 24.0 cc.

‡ Removed before addition of 7.0 cc.

45 per cent of the total base concentration. In the remaining seven experiments where total base decreased, the sum of the bile salt and undetermined anion concentrations was less than 45 per cent of the total base concentration. There appears to be an approximately linear relationship between changes in total base and the ratio of the bile salt plus undetermined anion concentration to the total base; as the bile salt plus undetermined anion comes to balance a larger proportion of the total base, decrease in total base concentration gives way to increase.

Since bile salt and undetermined anion tended to increase in concentration we felt it advisable to conduct experiments in which we could expect

maximum concentrating effects and still have sufficient material for analysis. After preparation as previously described known amounts of hepatic bile were added at intervals to the gall bladder. Specimens just sufficient for analysis were removed at intervals and further additions of hepatic bile continued. These experiments were carried over a period of from 24 to 48 hours. When this was done the results were different than those obtained in the short experiments (table 4).

In these experiments chloride and bicarbonate concentrations were reduced while calcium, bile salt and total base concentrations were constantly increased as long as the gall bladder remained normal. In each instance the distribution of the electrolytes was similar to that found in the gall bladder bile removed at operation. In one instance the activity was such that the bile removed after a period of study was greater in calcium and bile salt and less in chloride concentration than that originally removed from the same gall bladder. In every instance the equivalent

TABLE 5

Depression of the freezing point

DOG NO.	BASE	BILE SALT	CALCIUM	CHLORIDE	CO_2	CHOLESTEROL	Δ
			Hepatic bile				
	m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*mgm./100 cc.*	
535	174	42.0	3.4	55.0	64.8	11.7	0.537
			Gall-bladder bile				
494	325	343	30.1	1.4		100.0	0.563
446	287	289	27.6	4.7		29.0	0.576

concentration of total estimated acid radicles exceeded the equivalents of total base, a condition which we encountered in only one instance of all the hepatic biles examined. A very similar condition was found in the gall-bladder bile removed at the time of operation.

Osmotic pressure of hepatic and gall-bladder bile. Determinations of the depression of the freezing point were made with hepatic and gall-bladder bile. Table 5 gives the analysis of the bile specimens used. It is apparent that the osmotic pressure as calculated from the freezing point of hepatic and gall-bladder bile is approximately the same although the concentrations of the constituents of hepatic and gall-bladder bile are at wide variance.

Discussion. The observations reported in this paper were obtained from animals whose gall bladders were receiving known amounts of hepatic bile of determined concentrations. That the gall-bladder wall could function normally under the conditions of the experiment we had previously

determined. The maximum removal of water from the gall bladder we found to be 6.75 cc. per hour. These findings are similar to those of Brugsch and Horsters (1926) who found 6 cc. per hour as the maximum.

The variability of the anion—cation concentrations of hepatic bile in the same animal and in different animals may appear unusual. However, estimations of the pigment concentration of hepatic bile show even greater variations. (Rous and McMaster (1921), Hooper and Whipple (1916), and Riegel, Johnston and Ravdin, to be published.)

Jankau (1891–1892) found the calcium concentration of fistula bile of the dog to vary from 13 to 17 mgm. per 100 cc., and this was not increased by the oral administration of lime salts. Lichtwitz and Bock (1925) found that the calcium content of human fistula bile was from 65 to 84 mgm. per cent of the dry weight and from 85 to 109 mgm. per cent of the dry weight of gall-bladder bile. The calcium content per 100 cc. of fistula bile varied from 4 to 9 mgm. Since these observations were made on bile from exposed fistulae they are open to a number of criticisms. Drury (1924) stated that under ordinary circumstances when the animal was eating well the amount of calcium excreted per cubic centimeter of bile varied little from day to day and from animal to animal. The total calcium output per day varied considerably. Our animals were in good physical condition and ate the regular animal house mixed diet. Although as a rule the variation in the calcium concentration of hepatic bile was not great, low concentrations, considerably below the usual values for serum calcium, were occasionally found. Generally the concentration was above the level of the serum calcium, in some instances reaching twice that level. A study of Drury's data shows that he too found considerable variation in the calcium concentration of hepatic bile in that under normal conditions the concentration varied from 6.1 to 57.8 mgm. per 100 cc. Furthermore, the figures which he gives are as a rule above the concentration of calcium in the serum. We have never observed the high values which he occasionally found in hepatic bile. Whether the calcium is secreted at this high level or whether this might possibly result from the absorption of water by the hepatic ducts we do not know. We believe that the former explanation is more probably correct.

The bicarbonate concentration of hepatic bile was usually considerably higher than that of the serum. In the same animal there were variations of nearly 100 per cent. Drury, Rous and McMaster (1924) found the hepatic bile of the dog to be decidedly alkaline so that Gamble and McIver (1928) inferred that the hepatic bile contained a considerable amount of bicarbonate.

The pH of the hepatic bile of the dog, as determined with the glass electrode described by Stadie, O'Brien and Laug (1931) varied from 7.1 to 8.6±.01. The first studies which we have found in which the hydrogen

ion concentration of the bile is discussed were those of Brand (1902). Since his studies were done so long ago and were done on patients with biliary tract disease no analysis of his data will be given. He does, however, state that fresh liver bile is neutral or alkaline in reaction. Okada (1915) using an electrometric method found an average pH of 7.83 in the liver bile from 16 dogs. Neilson and Meyer, using a colorimetric method confirmed Okada's findings. Their principal studies were done on rabbit's bile.

The bile salts have shown a marked tendency to vary in concentration in different animals, but were fairly constant during similar conditions in the same animal. The chloride concentration varied although the variation was not so marked. The underlying causes for this variation in the anion concentrations is not clearly understood. Such fluctuations do not occur in the serum concentrations from hour to hour or from animal to animal. They may in part be related to the total bile output.

The fluctuation in the anion concentrations was reflected in the total base studies. Gamble and McIver (1928) reported variations in the base of from 157 to 194 m.Eq. per liter in the hepatic bile of the cat. We have found variations of base in the dog's hepatic bile from 174 to 192 m.Eq. per liter. No dry weight determinations were made and therefore no correction for water content of the bile was made.

The discrepancy between total base and determined anions of hepatic bile amounted at the median to 12 m.Eq. per liter. We have called this undetermined anion the "x" anion, realizing fully that it is probably made up of a number of anions. The amount of protein and phosphate present was very low but it is likely that pigment and fatty acids bind some of the excess base. Furthermore the observation that bile that has concentrated for a long period of time in the gall bladder contains more equivalents of acid radicles than of base renders it possible that even where determined anions are less than total base the total acid radicles may exceed the total base and hence the undetermined acid radicles which we have designated "x" anion may be greater than we have calculated.

When the hepatic bile was subjected to gall-bladder activity for a period of from 3 to 8 hours the bile removed was not uniform in the concentration changes which occurred. Base, chloride and bicarbonate generally decreased while pH always decreased. Calcium always, and bile salt generally, tended to increase in concentration. In those instances in which the change in the concentration of the substance was different from that usually attained subsequent experiments in the same animal showed that the results were not due to damage of the gall-bladder wall. We are unable adequately to explain the variations from the usual direction which have occurred. Westphal, Gleichman and Soika (1931) had similar discrepancies from what they considered the usual direction of change

when gall-bladder absorption was influenced by vagus or sympathetic nerve stimulation.

In the seven experiments where base decreased in concentration, anion also decreased. In the two experiments where base increased there was a decrease in the determined anion. The x or undetermined anion concentration increased in seven of the nine experiments. The median of the undetermined anion in these experiments was 26 m.Eq. per liter, an increase of 14 m.Eq. per liter over the median of the undetermined anion in hepatic bile.

Our results indicate that when the empty gall bladder received hepatic bile there occurred first a decrease in the concentrations of cation and total determined anion. During this period the bile salt concentration as well as the undetermined anion concentration increased. A relationship between the sum of the bile salt plus undetermined anion concentrations to the changes in base existed in every experiment When this ratio was below about 45 per cent total base decreased, but as the ratio approached the 45 per cent level total base again approached its original concentration and finally exceeded it. When these anions accounted for about 45 per cent of the base concentration total base built up. In all instances, however, even though the concentrations were increasing, there was absorption of base and anion when the total amount of base and determined anion in the gall bladder was calculated. This was also true for the individual anions. There was, however, a selective rate of absorption for the different anions, some leaving faster than water while others left at a slower rate.

When hepatic bile was added in small amounts at intervals to the normally functioning gall bladder for a period of from 24 to 48 hours, the samples removed showed a uniform change. Total base and bile salt concentrations increased while chloride and bicarbonate concentrations decreased. The bile removed from the gall bladder frequently contained more equivalents of acid radicles per cubic centimeter than of base. A similar result was obtained when the bile removed from the gall bladder at operation was analyzed. It was obviously impossible to calculate what the concentration of the x anion was in these specimens.

That other anions than those determined were present in small amounts we have shown in several instances. Some protein and phosphate was found, as well as a considerable amount of bilirubin. The results of Hammarsten (1929) indicate that fatty acids are present in hepatic bile and that these are markedly concentrated in gall-bladder bile. This finding probably accounts for a part of the increase of the x anion in the short experiments.

We can only speculate at this time as to the excess in equivalent concentration of acid radicles over base, when hepatic bile is permitted to concentrate in the gall bladder for a long period. The bile acid deter-

minations were expressed in equivalents of cholate measured colorimetrically against a standard of sodium cholate which was taken as monovalent. This calculation of the base binding equivalents of bile acids assumes that whatever the form of bile acids present the color development is proportional to the base binding equivalents. This must be regarded as a tentative approximation which further investigation must test.

The decrease of the pH of hepatic bile when subjected to gall-bladder activity is in accord with the recent observations of Okada (1915), Neilson and Meyer (1921), Drury, Rous and McMaster (1924).

The depression of the freezing point of hepatic and gall-bladder bile is significant. Our data indicate that both are approximately the same as that of serum. Coincident with the great increase in concentration of the bile salts there was no increase in the depression of the freezing point. In fact, the depression of the freezing point can be approximately accounted for by the osmolar concentration of the base, chloride and bicarbonate present. Our data are in agreement with those of Brand (1902) and Strauss (1903). Westphal, Gleichman and Soika (1931) give a depression of the freezing point of gall-bladder bile in the atropinized animal of $-0.55°C.$ and state that atropine does not prevent normal absorption. This figure is within the range which we have found.

SUMMARY

The base and the bicarbonate concentrations of hepatic bile are higher than that of serum and the chloride concentration is less than that of serum. Calcium concentration varies but as a rule it is higher than that of serum. The cations and anions all vary in concentration during different periods in the same animal. Chloride and bicarbonate show the greatest variations.

Gall-bladder bile has a higher concentration of base, bile salts and calcium and a lower concentration of bicarbonate and chloride than hepatic bile. The pH of gall-bladder bile is, under normal conditions, always lower than the pH of the hepatic bile brought to it.

Calculated for total amounts both base and the determined anions are absorbed from the gall bladder but the rate of absorption of the different ions varies considerably.

The depression of the freezing point of both gall-bladder and hepatic bile is approximately the same as that of serum so that even though there is a considerable difference in equivalent concentration of solutes present the osmotic pressure remains unaltered.

BIBLIOGRAPHY

BRAND, J. 1902. Pflüger's Arch., xc, 491.
BRUGSCH, T. AND H. HORSTERS. 1926. Arch. f. Exper. Path. u. Pharm., cxviii, 292.
DRURY, D. R. 1924. Journ. Exper. Med., xl, 797.

DRURY, D. R., P. D. MCMASTER AND P. ROUS. 1924. Journ. Exper. Med., xxxix, 403.
GAMBLE, J. L. AND M. A. MCIVER. 1928. Journ. Exper. Med., xlviii, 849.
HAMMARSTEN, O. 1929. Quoted by L. LICHTWITZ: Handbuch der Normalen und Pathologischen Physiologie, Berlin, iv, 608.
HOOPER, C. W. AND G. H. WHIPPLE. 1916. This Journal, xl, 332.
JANKAU, L. 1891–1892. Arch. f. Exper. Path. u. Pharm., xxix, 237.
JOHNSTON, C. G., I. S. RAVDIN, J. H. AUSTIN AND J. L. MORRISON. 1932(b). This Journal, xcix, 648.
LICHTWITZ, L. AND F. BOCK. 1915. Deutsch. Med. Wochenschr., xli, 1215.
NEILSON, N. M. AND K. F. MEYER. 1921. Journ. Inf. Diseases, xxviii, 510.
OKADA, S. 1915. Journ. Physiol., l, 114.
RAVDIN, I. S., C. G. JOHNSTON, J. H. AUSTIN AND C. RIEGEL. 1932(a). This Journal, xcix, 638.
RIEGEL, C., I. S. RAVDIN AND C. G. JOHNSTON. 1932(c). This Journal, xcix, 656.
ROUS, P. AND P. D. MCMASTER. 1921. Journ. Exper. Med., xxxiv, 47.
STADIE, W. C., H. O'BRIEN AND E. P. LAUG. 1931. Journ. Biol. Chem., xci, 243.
STADIE, W. C. AND F. W. SUNDERMAN. 1931. Journ. Biol. Chem., xci, 217.
STRAUSS, H. 1903. Berl. Klin. Wochenschr., xl, 261.
WESTPHAL, K., F. GLEICHMAN AND G. SOIKA. 1931. Pflüger's Arch. ccxxvii, 204.

SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

IX. Uric and Lactic Acids

A. K. SAIKI, G. OLMANSON and G. A. TALBERT

From the University of North Dakota, Grand Forks

Received for publication December 31, 1931

Adler (1) in 1916, using the Herzfeld colorimetric method, reports quite an appreciable amount of uric acid in the sweat, which is perceptibly increased when the subject is fed on a rich nuclein diet, his figures showing from 3 to 30 mgm. per 100 cc. of sweat. On the contrary Voit (2), using the method of Morris (3), finds there is something under 1 mgm. per 100 cc.

The insufficient amount of data and the wide divergence in the reports of the above named authors have prompted us to make a more exhaustive study of the problem, and as in our previous work we made simultaneous determinations of the uric acid in the blood and urine previous to and after the sweating.

The method of preparing the subject and the means of collecting the sweat, urine, and blood are the same as previously reported by Talbert (4) and his collaborators.

The colorimetric methods of Folin (5), Benedict (6) and Brown (7) were used at different times for making our determinations. All of these methods are essentially the same in that they all depend on phosphotungstic acid for color reaction. However, we found that the Benedict method, as reported by others, gives a slightly higher reading. In the sweat determinations the method of Folin and Brown was used. In the urine determinations we employed the Benedict; while in the blood determinations the Brown method was generally used.

For the determinations in the sweat no dilutions were made other than what the actual analysis entails. We did find it necessary, however, to centrifuge the sweat samples from 5 to 10 minutes at a rate of 2000 r.p.m. to delay precipitation long enough to permit a reading. The values for urine are given in milligrams per 10 cc., for blood in milligrams per 100 cc., and for sweat in milligrams per 1000 cc.

In all ninety observations of uric acid were made of which a few typical

examples are given in table 1. The blood taken after sweating, compared with the control sample, showed an increase in uric acid in 85 per cent of the cases, ranging from 1 to 30 per cent. The urine likewise in 85 per cent of the cases showed an increase in uric acid from 2 to 200 per cent. In thirteen cases, after sweating, samples of urine were taken at hourly intervals until 3 samples were voided. In nine of these cases this increase was maintained for one hour after sweating, and in three of them until the third hour.

This general rise in blood uric acid after profuse sweating may be due to the concentration of the blood, which we have previously shown to occur, rather than to an actual increase of uric acid. But we are disinclined to believe that the slight shift in specific gravity previously noted is a factor of sufficient magnitude to account for the results we have reported. Rather we would stress the rise of body temperature which uniformly obtained in such experiments as the major cause.

TABLE 1

Uric acid in blood and urine before and after sweating

SUBJECT	1ST BLOOD	2ND BLOOD	1ST URINE	2ND URINE	SWEAT
F. B.	2.13	2.13	4.42	3.45	1.19
J. L.	2.12	2.22	3.45	4.13	2.12
F. H.	2.57	3.42	5.68	6.17	1.09
F. C.	3.22	3.81	1.42	3.70	1.39
I. R.	4.03	4.17	2.02	6.94	2.00

It is worthy of note that we found very little uric acid in sweat. Our determinations ranged from 0.72 to 2.52 mgm. per 1000 cc. This is in close agreement with the results reported by Voit. On the other hand they disagree with those of Adler but since our subjects were not on a high nuclein diet they cannot be interpreted to contravene Adler's claim that sweating is a valuable therapeutic measure in certain clinical conditions.

Lactic acid may be found in significant amounts in sweat. In eighty-five observations on twenty subjects we never failed to obtain positive results using the method of Friedemann, Cotonio and Shaffer (8). Typical examples from our data, expressed in milligrams per 100 cc. are: 82.05, 81.36, 71.23, 160.45, 73.46.

SUMMARY

1. Uric acid is constantly excreted in the sweat in small amounts.

2. The uric acid content of both blood and urine rises after sweating. Rise of body temperature rather than blood concentration is preferred to explain this condition.

3. Lactic acid is constantly excreted in the sweat in appreciable quantities.

BIBLIOGRAPHY

(1) ADLER, A. E. Deutsch. Arch. f. Klin. Med., 1916, cix, 548.
(2) VOIT, K. Arch. f. exp. Path. u. Pharm., 1926, cxvi, 321.
(3) MORRIS, J. L. AND A. G. MACLEOD. Journ. Biol. Chem., 1922, l, 55.
(4) TALBERT, G. A. This Journal, 1931, xcvii, 426.
(5) FOLIN, O. Journ. Biol. Chem., 1922, liv, 159.
(6) BENEDICT, S. R. Journ. Biol. Chem., 1922, li, 187.
(7) BROWN, H. Journ. Biol. Chem., 1926, lxviii, 123.
(8) FRIEDEMANN, COTONIO AND SHAFFER. Journ. Biol. Chem., 1927, lxxiii, 331.

A STUDY OF THE EXCISED UTERUS OF THE RAT, ITS VOLUME DISPLACEMENT AND IRRITABILITY TO PITUITRIN, WITH REFERENCE TO THE OESTROUS CYCLE

O. G. HARNE

From the Department of Physiology, University of Maryland

Received for publication February 2, 1932

In a previous paper (1) we showed a definite relationship to exist between the stages of the oestrous cycle and the spontaneous activity of the excised uterus of virgin rats. Long and Evans (2) measured the horns in different stages and found the diameter to be greatest (5 mm.) in oestrus and least (1.7 mm.) in dioestrus. Osterud (3) weighed the different parts of the reproductive tract of animals of different ages and found that the uterus increased in weight consistently from birth to the 81st day, after which wide variations existed. (These variations occur normally during the sexual life of rats—Long and Evans, 1922.) We (1) have pointed out that the type of response obtained after pituitrin depends upon the pre-existing type of activity, whether normal or artificially induced, i.e., if the rate of contraction is slow, pituitrin (in minute doses) increases it, if maximum rate already exists, the effect is an immediate increase in tonus. These varying degrees of activity are described by Frank, Bonham and Gustavson (4) as resulting from the influence of the female sex hormone. Since this hormone is inhibitory in action (4) (5) we must deduce that its absence would result in a hyper-activity, and vice versa. It is apparent that something, exerting influence upon the irritability of the uterus to pituitrin, accompanies the cyclic changes. This will be discussed more fully presently. Durant and Rosenfeld (5) confirm Frank and his collaborators and report the effect as being primarily upon the rate and not the amplitude of contraction. Childs (6) suggests that the difference in activity found in isolated portions of the horns may be due to a gradient of metabolism. Experimental evidence following up this suggestion has not yet appeared; however, some recent work on the metabolism of whole excised horns was reported by Khayyal and Scott (7) who found active metabolic processes in the excised uterus of both the rat and the mouse, the oxygen consumption being greatest before oestrus, and constant at a lower rate during dioestrus. The mechanism controlling these metabolic changes points to the hormones associated with oestrus. When oestrus is artificially induced the oxygen consumption increases as oestrus ap-

proaches (7). The relation of oxygen consumption to spontaneous activity appears quite definite in dioestrus, but not so convincing in the first stages of the cycle. This deviation may be accounted for in the fact that in stages I and II uterine secretion raises the oxygen requirements. On this phase of uterine function more work is needed.

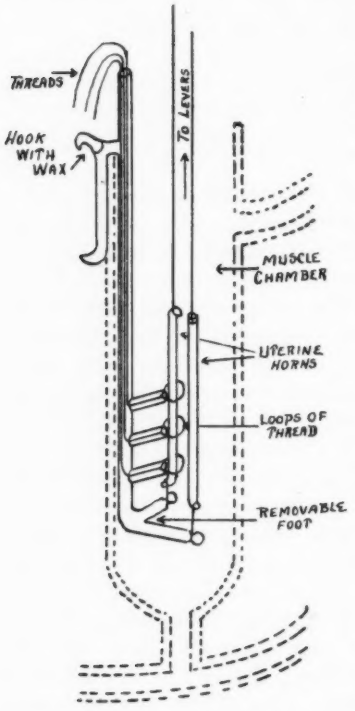


Fig. 1. Showing the system of ligation used. One horn is made to contract through a series of loops, each communicating with the outside, and may be tightened at will. The other is free.

In this paper it is desired to carry these investigations farther and study the uterine activity as it relates to volume displacement, relative and absolute length of horns, surface area and the influence of end-portions of the horns, upon spontaneous activity. Pituitrin in minute doses (0.01 cc. in 50 cc. of Locke's solution) is used to test the irritability. A system of ligation (fig. 1) is used making possible manipulation of the tissue at any time during the experiment.

Apparatus. The apparatus previously described (1) was used throughout with the addition of an especially adapted foot facilitating manipulation (see fig. 1).

Animal material. All animals were virgins in good condition and were selected for age and weight.

Procedure. The following additions to our original (1) were made. 1. Whole horns were first tied off at both ends, then cut free of their attachments and removed to the apparatus of Rubinstein (8) containing Locke's solution for volume displacement determinations, after which they were immediately transferred to the muscle chamber. 2. During the interval of relaxation (i.e., between contractions) the horns were measured under the stress of the writing lever (1 gram). 3. The tissues were given 30 minutes to establish their typical responses. 4. Finally, after the tissues had been experimented upon they were returned to fresh oxygenated Locke's solution and subjected to 5 times the stress of the lever (5 grams) and again measured for absolute length.

EXPERIMENTAL. For convenience these experiments may be divided into 3 groups: 1. The influence of ligating end-portions of the horns on spontaneous activity. 2. The relation of volume displacement and surface area to spontaneous activity. 3. The relation of volume displacement to pituitrin action.

1. *The influence of ligating end-portions of the horns upon spontaneous activity.* It is well known that if the uterus is bisected the two portions will contract independently but at different rates (1), (5). The ovarian end more closely represents the type of activity predicted for the whole horn (1). All these responses, however, are influenced by the oestrous cycle (1). One question arising out of recent observations we have attempted to answer here, viz: Where does the influence of the tubal end stop? In figure 2 the activity of 8 horns is reproduced, 4 exhibiting activity typical of the stage in which they were excised, and 4 sister horns subjected to various types of ligation. In curve I, 1 cm. of the vaginal end was ligated. Following one powerful response, irregular but otherwise typical contractions occurred accompanied by the characteristic fall of the tonus line. One may consider it a curve of reduced activity due to removal of a portion of the contractile tissue. In curve II a 3 mm. portion of the tubal end of the right horn was ligated before removal, while the left horn was kept intact. Over a period of 30 minutes no maximal contractions occurred in the ligated horn, while the normal (left) executed the characteristic responses. In curves III and IV the horns were removed and suspended in the usual fashion, and allowed to start their typical response, after which they were ligated to different extents, and at different time intervals. It was found that following this treatment inhibition resulted, the duration of which showed a definite relation to the oestrual stages. In the pre-oestrual stages (lt. V, I and ea. II) the inhibition may last a long time (30 minutes), while in the more active stages (III, IV, and ea. V) it is quite transitory, seldom lasting longer than 5 minutes.

The recovery from ligation and the type of return contraction are influenced by the amount of tubal end involved. In stages I and II characteristic inhibition can be induced by ligating very small portions (3 mm.), while in the more advanced stages 1 cm. may be required (curves II, III and IV, fig. 2).

The return contractions are difficult to analyze. They are never like the original in either rate or amplitude, but irregular, and occasionally compound in nature. They present possibilities, however, in that their normal controlling mechanism is apparently absent, and in this way more completely susceptible to environmental influences.

The tubal end influence may be completely removed in any stage of the oestrous cycle by ligating 1 cm. of the horn. The remainder of the horn will respond in a manner typical of the vaginal end of the uterus.

These experiments point to the possibility that the irregular curves often observed may be the result of ligation or destruction of the tubal

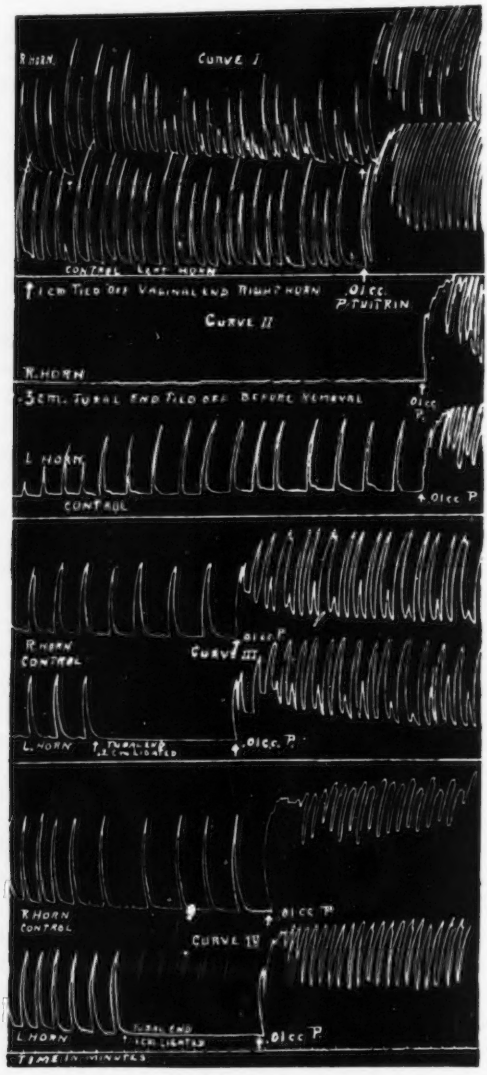


Fig. 2. Showing normal responses, and effect of various types of ligation upon 4 pairs of horns from virgin rats of equal size, and condition. The records of each pair were made simultaneously.

end of the horn, and suggest that the tubal end may exert upon uterine activity a controlling influence, which if cut off results in irregularity of response, and a reduced rate of contraction.

2. *The relation of volume displacement and surface area to spontaneous activity.* The curve in figure 3 represents the volume displacement of whole uterine horns of animals of approximately the same weight (178 grams, plus or minus 5 grams), excised at various stages of the oestrous cycle. The uterus was found to be large during oestrus and small in dioestrus, confirming Long and Evans (2). The change from one level to the other is relatively abrupt, but more pronounced during regression.

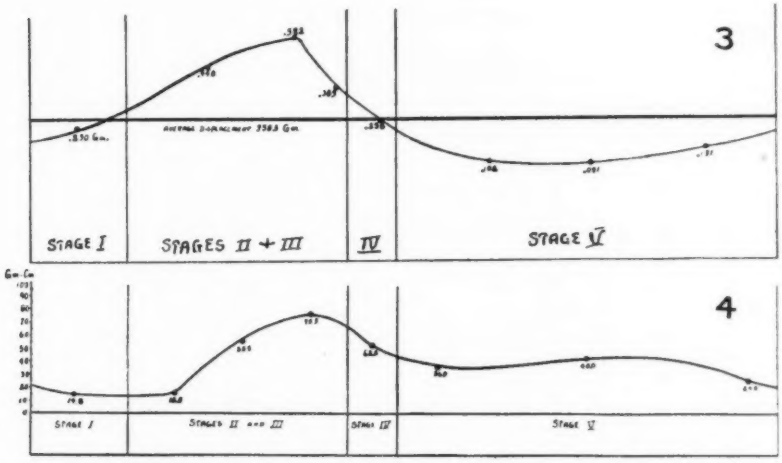


Fig. 3. Showing volume displacement of left uterine horns of virgin rats with reference to the oestrous cycle. Weight 178 grams (plus or minus 5 grams). Vertical lines divide the stages.

Fig. 4. Showing the curve of uterine activity, expressed in gram-centimeters of work, for left horns of virgin rats weighing 165 grams (plus or minus 3 grams) with reference to the oestrous cycle.

The average displacement (0.260 gram of Locke's solution at 25°C.) is present only momentarily, occurring in stage I and again in stage IV or early V. The displacement of the uterus is above average for about $\frac{1}{3}$ (30 hours) of the oestrous cycle (stages I, II, III and IV) and below average about $\frac{2}{3}$ (70 hours) of the cycle (stages IV, V and early I). These changes in volume displacement became interesting when compared with the curve of uterine activity.

In figure 4 the curve of spontaneous activity is plotted from left horns of 8 animals (nos. 2, 3, 5, 6, 10, 12, 14, and 16, table 1) upon the same scale of hours as figure 3. The activity is expressed in gram-centimeters of

work per hour. The curves practically coincide in the ascending phase, but diverge as they descend. We must deduce from this that while there is regression taking place there is also a force maintaining spontaneous activity, but at a submaximal level. The curves, which in stage II rose together, take opposite directions for a period in dioestrus. This means that volume displacement or tissue mass cannot account for the varying degrees of activity observed in the different stages of the oestrous cycle, and points to other phenomena.

TABLE 1

Showing the relation between length of horns, volume displacement, and work, with reference to the oestrous cycle

ANIMAL NUMBER	WEIGHT	STAGE	LENGTH OF HORNS 1 GRAM STRESS		ABSOLUTE LENGTH OF HORNS 5 GRAMS STRESS		VOLUME DISPLACEMENT		WORK	
			Left	Right	Left	Right	Left	Right	Left	Right
	grams		*cm.*	*cm.*	*cm.*	*cm.*	*grams*	*grams*	*grams-cm.*	*grams-cm.*
1‡	173	I	4.2	4.3	4.3	4.4	0.230	0.259	21	21
2§	164	I	4.0	4.2	4.1	4.3	0.222	0.230	14.8	17
3§	168	II Ea.*	4.2	4.2	4.3	4.5	0.450	0.474	12	12
4‡	178	II	4.2	4.4	4.3	4.5	0.440	0.445	31	34
5§	168	II	4.0	4.5	4.2	4.6	0.563	0.574	55.5	63
6§	164	III Ea.	3.9	4.2	4.1	4.4	0.574	0.570	75.3	81
7‡	183	III	4.2	4.3	4.4	4.4	0.582	0.580	70	72
8‡	180	III Lt.†	4.2	4.1	4.4	4.3	0.385	0.378	50	46
9‡	183	IV	4.5	4.6	4.7	4.7	0.255	0.255	54	57
10§	162	IV	3.9	4.1	4.3	4.5	0.269	0.267	62.5	60
11‡	184	V Ea.	4.1	4.3	4.3	4.5	0.102	0.101	39	40
12§	162	V Ea.	3.7	4.0	3.9	4.2	0.100	0.108	36	40
13‡	182	V	4.1	4.3	4.3	4.6	0.091	0.102	38	40
14§	164	V	3.9	4.0	4.3	4.5	0.081	0.090	40	40
15‡	178	V Lt.	4.1	4.3	4.3	4.6	0.131	0.120	32	32
16§	164	V Lt.	3.8	3.8	4.1	4.2	0.140	0.154	24	25

* Ea. = Early.

† Lt. = Late.

‡ Animals used in plotting the curve of volume displacement.

§ Animals used in plotting the curve of uterine activity.

3. *The relation of volume displacement to pituitrin action.* From the beginning we noticed that the uterus of certain animals of our series was sensitive to minute doses (0.01 cc. in 50 cc. of Locke's solution) of pituitrin, while others tolerated that amount with little evidence of the typical effect. Three possibilities present themselves. 1. The surface area exposed to pituitrin was not always the same. 2. The irritability of the uterus may follow the curve of spontaneous activity. 3. The activity may be controlled by a hormone (4).

Toward the solution of these problems additional experiments were performed and our older experiments again analyzed. Horns of the same absolute length (measured under 5 grams stress after the experiment), but in different stages of the oestrous cycle were selected from the lot for analysis here (animals 1, 4, 11 and 15, table 1). The results are plotted in figure 5. It was found that while the maximal height of contraction is maintained throughout stages I, II, III, and IV (C, fig. 5), in stage V it is decreased per unit length of horn. This result is so consistent in stage V that we must attribute it either to some inhibiting process, or to the absence of an activating substance. Our experiments point to the former.

When these horns are subjected to small doses (0.01 cc.) of pituitrin the response is entirely independent of tissue mass or surface exposure.

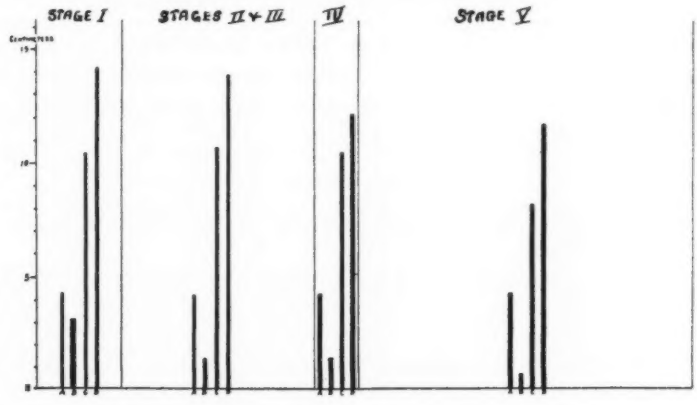


Fig. 5. Showing the relation of spontaneous activity of excised uterine horns of virgin rats, before and after small doses of pituitrin "O" (0.01 cc.) to the stages of the oestrous cycle. A, length of horn; B, tonus rise after pituitrin; C, normal height of maximal contractions; D, height of maximal contraction after pituitrin (0.01 cc.).

In stage I, where the volume displacement is average or below, the response is greatest (B and D, stage I, fig. 5). In stages II and III the displacement is far above average, but the effect upon tonus is much reduced (B, stages II and III, fig. 5). Stage IV presents an additional variation—the rise in tonus remains, but the tissue does not contract to a maximum (D, stage IV, fig. 5). A combination of these effects is found in stage V. The tonus increase is slight. The ability of the tissue to shorten is increased, but is still incomplete. As this stage approaches stage I, the tonus is much increased, and the contractions become maximal. In midstage V it seems that the pituitrin action is chiefly expressed in the building up of stunted maximal contractions. From the gross aspect it seems possible that the difference may be due to the small surface area exposed to the

drug, but this is dispelled upon closer analysis. If the dose of pituitrin be increased by 0.001 cc. (10 per cent, all the characteristic responses return, the tonus rise is increased, and the horns completely shortened. Since the volume displacement in stage V is often only $\frac{1}{6}$ and the surface area 32 to 40 per cent of that in stage II, the slight increase in dosage would be ineffectual if either surface area or volume displacement was a chief factor. It seems more probable that there exists some substance the function of which results in the volume changes observed, and bears a definite relation to pituitrin action and oestrual changes. The presence of this substance produces uterine inhibition with a low threshold to pituitrin, and vice versa. It is only by this premise that we can explain our results, and correlate the evidence at present in hand. This substance is in its highest concentration in stage I and gradually diminishes through the succeeding stages to late stage V where it shows recovery, and the cycle repeats. Upon the activity of this substance the curves of uterine activity, volume displacement, oxygen consumption, and the variable response to pituitrin can be explained and correlated with respect to the cyclic function of the reproductive tract.

CONCLUSIONS

1. Volume displacement of uterine horns has no certain relation to spontaneous activity.

2. The volume displacement of horns of virgin rats weighing 173 grams (plus or minus 11 grams) ranges between 0.081 gram in stage V to 0.582 gram in stages II and III.

3. The variation in the response of excised uterine horns to small doses of pituitrin cannot be explained on the basis of surface area exposed.

4. There exists in excised uterine horns of the virgin rat a graded irritability to pituitrin. The irritability decreases as the stages advance, i.e., it is highest in stage I and lowest in stage V.

5. Irregularity of response in excised horns is doubtless due to injury or absence of the tubal end of the horn. In stage III the influence of ligation is least, while in stage I it produces long intervals of inhibition.

BIBLIOGRAPHY

(1) Harne, O. G. This Journal, 1931, xcix, 227.
(2) Long and Evans. Memoirs Univ. California, 6, Univ. Calif. Press, 1922.
(3) Osterud, H. L. Proc. Amer. Assoc. of Anatomists, Anat. Record, xxi, 75.
(4) Frank, R. T., Bonham and R. S. Gustavson. This Journal, 1925, lxxiv, 394.
(5) Durant and Rosenfeld. This Journal, 1931, xcviii, 153.
(6) Childs, C. M. This Journal, 1917, xliii, 87.
(7) Khayyal, M. A. and C. M. Scott. Proc. Physiol., Soc., May 23, 1931, Journ. Physiol., lxxii, no. 4, 13.
(8) Rubinstein, H. Manuscript.

STUDIES ON THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

XI. Effect of Crotalin on Swine

HIRAM E. ESSEX

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

Received for publication January 22, 1932

In many localities the belief is current that swine possess a natural immunity to the venom of poisonous snakes. It is claimed by many farmers that rattlesnakes will not live in fields frequented by hogs since the latter attack snakes, kill and eat them, and that hogs are not harmed since they are immune to the venom of the snake. The desire to test the validity of such statements prompted this study.

Previous studies have shown that the injection of crotalin into dogs and rabbits produces a marked fall in blood pressure and causes a decided increase in the volume of the erythrocytes in vivo or in vitro, accompanied by more or less hemolysis. The susceptibility of a given animal to crotalin may be judged by the response of the animal's blood pressure and erythrocytes to a standard dose of this venom. The effect of crotalin on swine is illustrated by the following experiments.

A shoat, aged five weeks, weighing 6.7 kgm., was etherized and the carotid blood pressure recorded in the usual manner. An intravenous injection of 0.03 cc. of 2 per cent crotalin (Crotalus horridus) for each kilogram of body weight, which is about half the usual lethal dose for the dog, was made into a vein of the ear. Immediately following the injection the blood pressure fell rapidly from 100 mm. to 30 mm. of mercury. Twenty minutes later it began to rise and had reached a physiologic level in about thirty minutes. The same dose was repeated and a similar response resulted but recovery was delayed. Twenty-four minutes after the second injection the blood pressure had risen to only 50 mm. of mercury where it remained until the tracing was discontinued fifty minutes later (fig. 1). The shoat gradually recovered and was walking about and taking food sixteen hours after the experiment

To determine the effect of the venom on the erythrocytes, a sample of blood (11 cc.) was drawn into heparin immediately before the first injection and six minutes after the second injection. These specimens were centri-

fuged for twenty minutes at about 1,500 revolutions each minute. The volume of the erythrocytes in the first tube was 5 cc. and there was no hemolysis, whereas the volume of the erythrocytes in the second tube was 8.2 cc. and considerable hemolysis was present. As is true with the dog the clotting time of the blood of swine is prolonged following an injection of crotalin. In one instance the blood was still fluid after sixteen hours.

Twelve days after the experiment described, the shoat was given an intravenous injection of 1 mgm. of dried venom for each kilogram of body weight. This was followed almost immediately by complete prostration. The breathing was very shallow, the pulse was rapid and thin. The symptoms differed from those of the dog since there was absence of vomiting, urination and voiding of bloody feces. Within an hour the shoat was walking about apparently recovered. The next morning, however, it was found dead. At necrospy the jejunum was full of blood. The lungs were

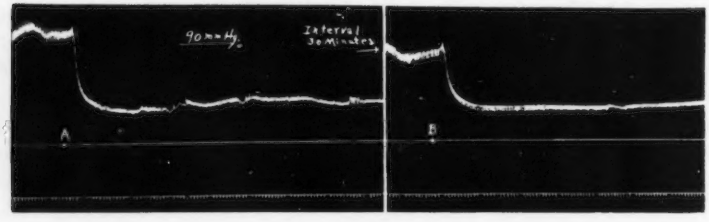


Fig. 1 *a*. The results of two successive intravenous injections, A and B, of 0.03 cc. of 2 per cent rattlesnake venom for each kilogram of body weight in a pig weighing 6.7 kgm. Time in intervals of five seconds.

distended and the heart showed a few petechial hemorrhages in the auricles, and in the ventricles were areas of subendocardial hemorrhage near the bases. As occurs in the dog, the bladder was extremely contracted. The blood in the heart and large vessels was fluid. Similar results were obtained in other experiments. In one instance a shoat succumbed to a dose of 0.03 cc. of 2 per cent crotalin for each kilogram of body weight but in all other experiments a much larger dose was required to produce death. An intravenous injection of 1 to 1.5 mgm. of the dried venom for each kilogram of body weight might be expected to prove fatal in a high percentage of cases.

COMMENT

The physiologic responses of the pig are similar to those of the dog following an intravenous injection of a lethal dose of crotalin. The characteristic fall in blood pressure, accompanied by a decided increase in the

volume of the erythrocytes, and the post-mortem observation, resembled closely those noted in other susceptible animals.

It is probable that the thick layer of subcutaneous fat usually possessed by swine may sometimes retard rapid absorption of the venom following a bite, and thus acute poisoning may be prevented. However, it is safe to conclude in the event a hog is bitten by a poisonous snake, serious symptoms may reasonably be expected to follow if a sufficient quantity of venom has been injected. It is apparent that swine are not more immune than other domestic animals to rattlesnake venom.

THE FAILURE OF DIET TO INFLUENCE THE ADRENIN CONTENT OF THE ADRENAL GLANDS

ARTURO ROSENBLUETH[1] AND RENÉ GAYET[2]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 3, 1932

Cannon and Uridil (1921) found in cats a greater output of a cardio-accelerator substance, when they stimulated the nerves of the liver, if the animals were digesting protein. Since adrenin, sympathin, and the substance liberated from the liver have similar physiological effects (Rosenblueth and Cannon, 1932), it was deemed interesting to learn whether diet influences the adrenin content of the adrenals.

Rats were chosen because they are omnivorous. Lots varying from 5 to 15 were put on different diets for varying periods (8 to 35 days). Four lots received only lean meat and water as food; three others only water and beef-fat; three others only water and sugar; one lot, finally, had only water for eight days.

The animals were killed by a blow on the head. They were bled by cutting the carotids. The glands were immediately extirpated, weighed and ground with sand and hydrochloric acid. The adrenin content was determined by means of the colorimetric method of Folin, Cannon and Denis (1913).

The following results were obtained:

DIET	NUMBER OF DAYS	NUMBER OF RATS	WEIGHT OF RATS BEFORE DIET	WEIGHT OF RATS WHEN KILLED	WEIGHT OF ADRENALS	ADRENIN PER 1 GM. OF GLAND
			grams	*grams*	*mgm.*	*mgm.*
Protein	8	5	670	680	230	3.5
	8	11	1,980	2,028	480	3.3
	15	11	2,430	2,480	420	3.4
	35	10	1,030	1,318	350	3.9
Fat	8	5	670	660	110	2.6
	8	12	2,580	2,596	710	3.1
	35	13	1,482	1,600	500	3.6
Sugar	8	6	620	612	140	3.2
	8	11	1,710	1,668	470	4.1
	35	15	2,083	1,846	500	4.5
Fasting	8	7	1,087	1,027	310	1.8

[1] Fellow of the John Simon Guggenheim Memorial Foundation.

[2] Fellow of the Rockefeller Foundation.

These results show that diet has no consistent influence on the amount of adrenin in the adrenals of the rat.

The control observations on fasting animals confirm the results reported by Venulet and Dmitrowsky (1910), Ogawa (1925) and others, i.e., a lower content of adrenin.

BIBLIOGRAPHY

CANNON, W. B. AND J. URIDIL. 1921. This Journal, lviii, 353.
FOLIN, O., W. B. CANNON AND W. DENIS. 1913. Journ. Biol. Chem., xiii, 477.
OGAWA, S. 1925. Arch. f. Exp. Path. u. Pharm., cvii, 171.
ROSENBLUETH, A. AND W. B. CANNON. 1932. This Journal, xcix, 398.
VENULET, F. AND G. DMITROWSKY. 1910. Arch. f. Exp. Path. u. Pharm., lxiii, 460.

THE CURRENT FLOWING THROUGH THE HEART UNDER CONDITIONS OF ELECTRIC SHOCK

WILLIAM B. KOUWENHOVEN, DONALD R. HOOKER AND ORTHELLO R. LANGWORTHY

From The Johns Hopkins University

Received for publication January 12, 1932

In studying the effect of electric shock upon rats (1, 2, 3) it became evident that the injury was in the main confined to the portion of the body that was traversed by the current. The present paper is directed specifically to a study of the effect upon the heart since this organ is believed to be especially liable to injury in electric shock. The purpose of the present experiments was two-fold; to ascertain the proportionate value of the total current which actually flows through the heart when contact at various points on the body is made with the circuit and to determine the minimum current necessary to establish ventricular fibrillation.

Dogs, completely anesthetized with morphia and ether, were used as experimental animals because the ventricles of the dog's heart are readily thrown into a permanent state of fibrillation by the application of relatively weak currents as is assumed to be the case in man.

The source of the current was a 60 cycle, alternating current circuit. Ring or through type current transformers were employed to measure the current traversing the heart, the heart itself forming the primary winding of the transformer. The iron cores of these transformers were ring shaped and of the highest quality magnetic material.[1] They were approximately square, having a cross-sectional area of 0.01 square inch (0.07 sq. cm.). Each ring was uniformly wound with approximately 2000 turns of no. 36 Brown and Sharp enamel insulated copper wire which served as the secondary winding on the transformer. The rings were of varying inside diameters, ranging from $\frac{5}{8}$ inch up to $3\frac{1}{2}$ inches, so that they could be slipped over hearts of different sizes. The current flowing through the heart induces an electro-motive force in the secondary winding of the transformer encircling it. The terminals of this secondary winding were connected across a potentiometer type rheostat, and the desired portion of the voltage drop in this rheostat was fed into an amplifier whose

[1] The authors wish to take this opportunity to thank the Bell Laboratories Inc. and the Westinghouse Electric and Manufacturing Company for their kindness in furnishing the transformer cores.

output was a measure of the current flowing through the heart. The complete details of the electrical equipment are given in another paper by one of the authors (4).

In order to check the operation of the ring transformers they were immersed at the center of a conducting bath consisting of a ten per cent sodium chloride solution. The bath was over six feet in length and approximately five by five inches in cross-sectional area. In a bath of these dimensions, placing the electrodes at the extreme ends insures a uniform current distribution at the center. Tests proved that when the plane of the ring was perpendicular to the direction of the current flow, the amplifier reading measured the total current flowing through the area inclosed by the ring. When the ring was turned so that its plane was parallel to the current path the amplifier reading was zero. In all of the tests it was found that the amplifier output was equal to the current actually flowing through the area inclosed by the ring. Therefore, with the ring inclosing the heart it measured the current through that organ.

Application of transformer. The thorax was opened under artificial respiration and the pericardium freed from its attachment to the diaphragm. A ring transformer of suitable size was passed over the apex until it fitted snugly about the mid-region of the ventricles where it was held in place by ligatures passing through the pericardium. After the ring was in place the pericardium was sewed closely to its original attachment on the diaphragm. The fully insulated lead wires were carried to their proper connections and the thorax was then roughly closed, to establish *approximately* normal conditions.

The heart is such a mobile structure that it is difficult to determine its electrical conductivity at any single instant. Its only permanent connection is with the great vessels at its base. During expansion of the lungs they make good contact at the sides, a contact which is less perfect during expiration. The apex of the heart lies in proximity to the diaphragm but the effectiveness of contact at this point varies continually. Moreover the operation upon the chest caused a further change in these normally variable conditions. By closure of the chest after insertion of the ring and applying mechanical inflation and deflation of the lungs throughout the experiments, physiological conditions were preserved as carefully as possible.

The equipment controlling the voltage supplied to the electrodes was such that any desired value of voltage could be obtained and the total current passing through the body held constant. An ammeter inserted in the circuit measured the total current.

Period during which current distribution is constant. With different points of contact on the body the amount of current passing through the heart could be determined upon the animal after death provided no appre-

ciable change developed in the current distribution through the body. Figure 1 shows the results of one experiment. In this case the total current through the animal (head to tail) was held constant by controlling the voltage of the supply circuit. Artificial respiration was continued even after the heart ceased to beat and the current flowing through the heart varied slightly, presumably with the amount of air in the lungs, but it is apparent that satisfactory constant conditions prevail for at least thirty minutes.

With these data in hand, which were confirmed in other experiments, it was felt justifiable to continue observations on the dead animal for the period specified in order to conserve material.

In a number of experiments the total current flowing through the body was varied from a low value to several hundred milliamperes, and the current flowing through the heart noted. The heart current proved to be a fixed percentage of the total current flowing.

The change in the current distribution apparently coincides fairly closely with the onset of complete rigor mortis. It was noted in the experiments

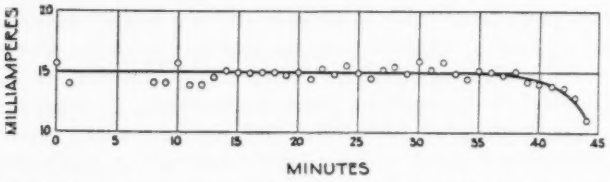


Fig. 1. Variation of current through heart with time following the death of the animal.

that rigor of the neck musculature was judged to be complete one-half hour after death.

Current passing through heart for different current paths. Tests were made on a number of animals with the ring transformers encircling the heart, thus measuring the current that flowed through the heart for various current paths. In these experiments the total current flowing through the body was varied in the different animals, but in any given animal it was held constant during the test. The electrodes were first placed on the head and tail. The total current through the animal was adjusted to the desired value and that through the heart read on the amplifier. The electrodes were then shifted to a new position, the total current through the dog readjusted to the given value and that through the heart measured. The electrodes were then shifted to the next position and the procedure repeated. When all the important current paths had been tried, the electrodes were shifted back to head and tail and a second series begun. It was possible to obtain four to five series of readings on each

animal during the half hour period before rigor mortis set in. Thus each value of heart current for a given current path found in table 1 represents the average of four or five separate readings taken at intervals of six or seven minutes. In some of the tests only a few current paths were studied and in these cases the data in the table are the average of a larger number of readings.

It is evident from table 1 that there is a fair agreement between the results obtained with the different animals when the current flowed downward through the trunk. However, for points of contact where the current path was at right angles or nearly so to the trunk the agreement is

TABLE 1

Ring encircling heart

	WEIGHT	TOTAL CURRENT	PERCENTAGE OF TOTAL BODY CURRENT PASSING THROUGH HEART FOR CURRENT PATHWAY FROM:												
			Head to tail	Head to right hind-leg	Head to left hind-leg	Right fore-leg to left hind-leg	Right fore-leg to right hind-leg	Left fore-leg to left hind-leg	Left fore-leg to right hind-leg	Right fore-leg to tail	Left fore-leg to tail	Right hind-leg to left hind-leg	Head to right fore-leg	Head to left fore-leg	Right fore-leg to left fore-leg
	pounds	*milli-amperes*													
1	8	160	9.0												
2	8	55	9.0												
3	9.5	160/235													0.8
4	9	150	7.7	8.4	8.8	—	—	6.6	6.4	—	6.9	0	5.5	3.9	—
5	10.7	150	8.5	10.1	10.0	6.8	7.1	8.8	8.5	7.0	8.8	0	3.1	1.0	1.3
6	9.3	100	8.8	8.7	8.3	7.5	7.7	6.3	6.5	7.5	6.3	0	6.6	0	6.7
7	7.5	150	7.5	—	—	6.2	6.2	—	—	7.0	—	0	3.9	1.1	4.7
8	11.0	150	9.7	10.8	9.8	8.3	8.3	8.0	8.6	7.7	7.6	0	0.5	0.5	0
9	11.3	120	10.5	10.4	10.3	9.5	10.3	5.5	5.3	9.1	4.7	0	0	4.3	3.9
Average.....			8.8	9.7	9.5	7.7	7.9	7.0	7.1	7.7	6.9	—	3.2	1.8	2.9

poor and there is a wide variation in the values obtained with different animals. The lack of agreement is probably due to several causes, the principal one being the difficulty of restoring complete normal conditions when closing up the opening in the chest cavity.

A study of the results in table 1 shows that approximately nine per cent of the total current flowing down through the trunk from the head to the lower extremities passed through the heart. It is interesting to note that more current flowed through the heart when the current entered the right fore-leg and passed out at either hind-leg or tail, than when it entered via the left fore-leg. These results check the earlier experiments (2) made on rats when it was found that only 33 per cent recovered when the current

path was from right fore-leg to tail, while 58 per cent recovered when the left fore-leg was used in place of the right. It was found that when the current path was from hind-leg to hind-leg, no current flow through the heart could be detected. This result also confirms experimental data found with rats (2).

The last three columns of table 1 give the data for the cases where the current pathway was from the head to either fore-leg or from one fore-leg to the opposite fore-leg. When contact at the body is made at these points the current flows more or less transversely through the thorax. As already pointed out, the agreement here is poor. A few experiments were made with the two inch ring transformer sewed along the right side of the heart, thereby fixing its plane parallel to the body axis. The current path in these tests was between the fore-legs and the average current flowing through the ring was found to be three per cent of the total current in the circuit. This value also checks closely that found with the transformers encircling the heart.

Current necessary to initiate fibrillation. In seven experiments the current through the heart necessary to initiate fibrillation was measured. The electrodes were attached to the head and tail of the animal, and the total current was gradually increased using a five second shock at each value. The current actually flowing through the heart for each shock was determined from the amplifier output. After each five second shock the thorax was opened and the condition of the heart visually determined. There was considerable variation among the individual animals as was to be expected. In one case a heart current of six milliamperes caused fibrillations, in another the value was 15 milliamperes. The average value was 8.6 milliamperes.

Fibrillation experiments. In order to check the accuracy of the results several tests were made to find the amount of current that must be applied to the surface of the body with different leads in order to produce ventricular fibrillation without the ring transformers in place. In these tests one electrode was kept permanently connected to the right fore-leg while the other was connected to either the left fore-leg or to the two hind-legs which were used as a single terminal.

Two experiments were made with the heart perfused *in situ*. With the current pathway down through the trunk, the current through the animal was increased until fibrillation was initiated. Then the heart was recovered by the injection of potassium chloride into the coronary arteries (5). Next the movable electrode was transferred to the left fore-leg and the current now flowing transversely through the body adjusted to the value that would initiate fibrillation for this current pathway.

The results of one of these experiments are given in table 2. These data show clearly that for this animal a total current of 90 milliamperes

flowing in at the right fore-leg and out at the lower extremities was enough to set the heart in fibrillation. With the current flowing transversely between the fore-legs it required 243 milliamperes to start fibrillation.

The data in table 1 gave the average heart current as 7.8 per cent of the total body current for a current pathway from the right fore-leg to either

TABLE 2

DURATION OF SHOCK	TOTAL BODY CURRENT	POSITION OF ELECTRODES	HEART RESPONSE
Blood flowing normally through heart, thorax open, artificial respiration			
seconds	*milliamperes*		
5	92	Right fore-leg. Left fore-leg	No fibrillation
5	88	Right fore-leg. Hind leg	Fibrillation. Heart recovered
Heart perfused with oxygenated Locke's solution. Artificial respiration continued			
5	85	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	91	Right fore-leg. Left fore-leg	No fibrillation
5	103	Right fore-leg. Left fore-leg	No fibrillation
5	125	Right fore-leg. Left fore-leg	No fibrillation
5	83	Right fore-leg. Hind leg	No fibrillation
5	100	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	110	Right fore-leg. Left fore-leg	No fibrillation
5	135	Right fore-leg. Left fore-leg	No fibrillation
5	176	Right fore-leg. Left fore-leg	No fibrillation
5	220	Right fore-leg. Left fore-leg	No fibrillation
5	243	Right fore-leg. Left fore-leg	Short fibrillation, then normal. Heart recovered
5	102	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	79	Right fore-leg. Hind leg	No fibrillation
5	83	Right fore-leg. Hind leg	Fibrillation. Heart recovered
Heart beat feeble			
5	155	Right fore-leg. Left fore-leg	No fibrillation
5	79	Right fore-leg. Hind leg	Fibrillation

hind-leg. In this experiment using the same current path it required 90 milliamperes flowing through the animal to initiate fibrillation. This was equivalent to a current through the heart of seven milliamperes. In the cases where the current path was from fore-leg to fore-leg the current

through the heart was approximately 3 per cent of the total current, which corresponded in this check experiment to 7.3 milliamperes through the heart. This test was successfully repeated several times and the current values check closely, as may be seen from table 2. The results obtained with the other animal tested in this manner were equally good.

SUMMARY

It is evident from these experiments that 9 or 10 per cent of the total current passing through the body flows through the heart for a current pathway parallel to the body axis. When the current flows transversely only about 3 per cent passes through the heart. Thus as far as the heart is concerned fibrillation will be produced by a much smaller total current flowing from the upper to the lower extremities than between the fore-legs.

In most industrial accidents the current path is from right hand to the feet and under these conditions the heart carries a greater proportion of the total current than when contact with the circuit is made at any other location on the body.

The authors wish to take this opportunity to acknowledge their appreciation to the Committee on Physiology of the Conference on Electric Shock for providing funds for this work.

BIBLIOGRAPHY

(1) KOUWENHOVEN, W. B. AND O. R. LANGWORTHY. Journ. A. I. E. E., 1930, xlix, 25; also Trans. A. I. E. E., 1930, xlix, 381.
LANGWORTHY, O. R. AND W. B. KOUWENHOVEN. Journ. Indust. Hyg., 1930, xii, 31.

(2) KOUWENHOVEN, W. B. AND O. R. LANGWORTHY. Trans. A. I. E. E., 1931, l, 1165.
LANGWORTHY, O. R. AND W. B. KOUWENHOVEN. Journ. Indust. Hyg., 1931, xiii, 145.

(3) KOUWENHOVEN, W. B. AND O. R. LANGWORTHY. Elec. Journ., 1931, xxviii, 472.
LANGWORTHY, O. R. AND W. B. KOUWENHOVEN. Journ. Indust. Hyg , 1931, xiii, 326.

(4) KOUWENHOVEN, W. B. Rev. Sci. Instr., 1931, ii, 541.

(5) HOOKER, D. R. This Journal, 1929, xci, 305.

THE DIGESTIVE LEUCOCYTOSIS QUESTION

W. E. GARREY AND VIRGINIA BUTLER

From the Department of Physiology, Vanderbilt University Medical School

Received for publication February 1, 1932

In 1854 Moleschott (1) described digestive leucocytosis which is said to be particularly noticeable after a meal of protein. Von Limbeck (2) asserts its existence, as do Arneth (3) and many others. The phenomenon has been uncritically accepted as a part of the physiologist's *credo*, but there have always been dissenters and in recent literature one frequently finds the interjection of a note of doubt (4, 5, 6). It may be questioned whether any really adequate investigation of the phenomenon has as yet been made owing to a lack of appreciation of the number of factors and the sensitiveness of the mechanisms which cause variations in the number of circulating leucocytes. The control of these variables is essential to a correct interpretation of experimental results and this is the objective in the experiments reported in this communication.

It is first of all necessary to establish an unvarying norm for the leucocyte count under conditions which permit the induction of one variable at a time, just as one compares all metabolic variations to the basal metabolic rate. Preliminary experiments (7) have demonstrated that the leucocyte count, made on blood obtained by deep, sharp puncture of either the finger or ear, of over two hundred students and staff members, pursuing ordinary laboratory activities, usually lies between 8000 and 9000 per cubic millimeter and this has been designated the *activity level*. The count may be somewhat higher or slightly lower depending on the individual and the degree of activity. Upon assuming the recumbent posture and maintaining a condition of absolute rest the count falls rapidly. In approximately an hour it reaches its lowest level which remains constant for hours as long as the state of rest and immobility are maintained. We designate this the *basal* leucocyte level. Numerically it usually lies between 5000 and 6000 leucocytes per cubic millimeter, although it varies somewhat with different individuals and occasionally may be somewhat above or below these figures (7, 8). A rise above the basal level constitutes a physiological leucocytosis and may be the accompaniment of extremely slight alterations of physiological state.

The first experiments were conducted on subjects in the basal metabolic state, in the early morning and without food. The results of six such

experiments are illustrated in figure 1, A and B; they are typical of all. Leucocyte counts were made at the activity level and every fifteen minutes after assuming the recumbent resting condition. After an hour when the basal level was reached a heavy protein meal was fed to the passive, immobile subjects. The meal consisted of one and a half to two pounds of beef steak, two to four eggs and one to two slices of toast, depending upon the capacity and willingness of the subject; no fluid was administered. The fifteen minute counts were continued for two to four hours—as long as the subjects could be kept quiet. There was not a single instance in which the leucocyte count rose above the basal level while the subject remained at rest. The authors consider that this constitutes a crucial experiment indicating that the processes of digestion and absorption *per se* do not cause a leucocytosis. Whenever, in the experiments under consideration, the subject assumed an erect posture there ensued an immediate

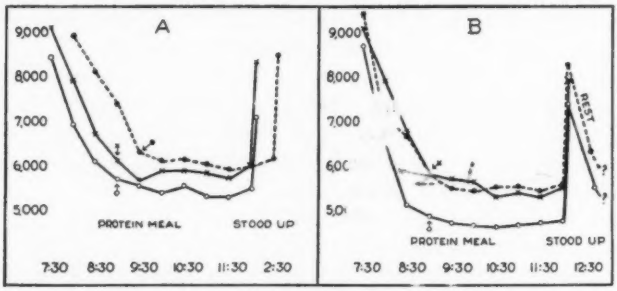


Fig. 1. Failure to obtain a digestive leucocytosis. Two groups of curves, A and B, three individuals in each group, showing 1, that recumbent rest decreases the leucocyte count to a basal level; 2, that a heavy protein meal, administered at the points marked by arrows, did not cause a rise in the basal level. The counts were made at fifteen minute intervals.

rise in the leucocyte count after which recumbent rest was followed by the gradual fall of count to the basal level again in spite of the progress of the digestive process.

In a similar way carbohydrate meals were fed, consisting of approximately five pancakes and about 150 cc. or 200 cc. of a well known brand of syrup. The test conducted as described for the protein meal gave an identical result and the graphs in figure 1 illustrate equally the effects of a carbohydrate meal. Details of the experiments are superfluous. There was no digestive leucocytosis.

A second type of experiment may be used to test the validity of the concept of digestive leucocytosis. The same criterion is used, viz., a change of the basal leucocyte count. Comparison is made of the basal

level of a given individual who has arrived at the laboratory in one instance without food and on the succeeding day having breakfasted. It has been found that the basal count of an individual who maintains a fairly regular regime will remain at a remarkably constant level day after day over a long period. The counts in these cases were made preferably in the early morning or fore-noon and with the assurance that there has been no infection, severe fatigue, loss of sleep or sustained emotional disturbance, otherwise variations such as those reported by Stetson (8), may creep into the picture. Such individuals appeared in the laboratory without breakfast (fig. 2, A) and on alternate days immediately after breakfasting. The basal level reached after an hour of recumbency was the same, irrespective of food intake on all days—allowance being made for a variation of three hundred cells either side of a mean, this variation being sometimes in the

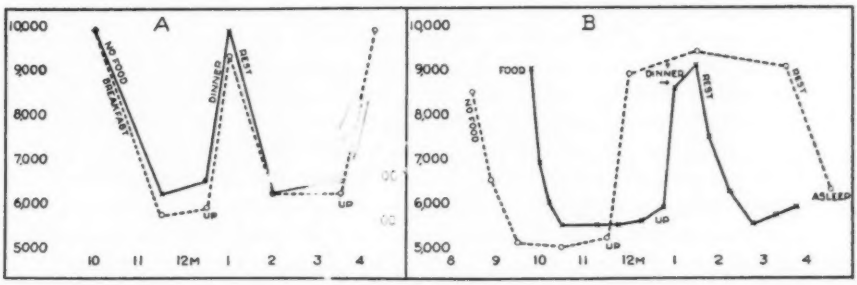


Fig. 2. A: Two leucocyte counts, made on successive days, on the same individual. Breakfast did not affect the drop to the basal level of recumbent rest; likewise a mid-day meal had no effect on the subsequent basal counts. B: Two individuals, one of whom had breakfasted; the basal leucocyte count was not affected by either the morning or noon-time meals. The changes recorded were due solely to posture and activity.

direction of increase in the count, equally often of decrease. Our records indicate this result upon eleven individuals in eighteen experiments. The average American breakfast therefore did not tend to increase the basal leucocyte count when precautions were taken to eliminate other causes of physiological leucocytosis.

The reaction to meals later in the day showed no deviation from the above findings—when two individuals, only one having breakfasted, appeared in the laboratory. There was a commensurate fall in the leucocyte count upon lying down until the individual basal levels were reached. Upon rising and going to a mid-day dinner the leucocyte count rose immediately to the activity level (fig. 2, B), but remained up only during the period of activity and fell according to rule upon lying down after the meal,

the rate and extent of the fall being independent of the preceding meals, although it should be noted in passing that the average afternoon basal count is sometimes slightly above that early in the day, a phenomenon which however is independent of meals and is irrelevant to this presentation. All of the evidence cited above indicates the absence of a true digestive leucocytosis; there is certainly no persistent rise of the basal count during the absorptive stage.

Since we had previously noted that localized peripheral stimuli acting reflexly may cause a transient rise in the leucocyte count, apparently by a vascular reaction, five series of counts were made with a view to determining the effects of gastric filling and temperature changes. Resting subjects with a basal count were given lemonade at different temperatures—approximately 5°C., 50°C. and 20°C., respectively. In each instance the subject drank to satiation—approximately three glasses. Counts made three minutes later showed a rise in every case, amounting with the cold solutions to a 15 per cent-30 per cent rise above the basal level. In the case of the hot drink the rise was higher, amounting to 22 per cent to 42 per cent of the basal count. In one instance the count was higher than the activity level, in all other cases slightly lower. It was further noted that within ten minutes the count was gradually falling so that the basal level was restored within half an hour. Water will cause the same reactions which seem to be related to the vascular alterations due to temperature changes and to the reflexes instituted by the acute gastric distention. Slow sipping of 500 cc. of water at room temperature caused no change in the leucocyte count. On the other hand a very small drink of water at body temperature induced nausea on three occasions, each accompanied by a sharp increase in the leucocyte count which is attributable to the vascular changes resulting from the emotional upset.

Animal experiments were resorted to in order to clarify the situation relative to gastric temperature and filling. Dogs were anesthetized with sodium barbital administered in solution through a stomach tube. The tube was later introduced and left in place to avoid the effects of repeated removal and reintroduction. The counts were made on blood drawn by deep puncture wounds of the upper lip, and checked with counts made on blood drawn from large veins. It should be stated that the counts on such animals remain constant for many hours, furthermore that spinal and medullary reflexes are extremely sharp and easily elicited (9). The results of the experiments are given in table 1 and may be summarized as follows: Rapid distention of the dog's stomach (350 cc.) by air or water at body temperature causes an instantaneous rise in the leucocyte count with a return to the initial level within a half hour. Similar quantities of fluid introduced at a slow rate do not affect the count. Either hot or cold water suddenly introduced have a similar but greater effect, the leucocy-

tosis persisting somewhat longer after the cold administrations. The promptness and transiency of these reactions place them definitely into the category of nervous reflexes affecting the vascular area (7, 9), and producing a sudden redistribution of leucocytes, with more gradual readjustment to normality. The rôle of the sympathetic nervous system has already been demonstrated by the authors (10).

Identical reactions of human subjects emphasize the limitations of the rôle which prandial libations can play in causing a rise in the leucocyte count. It is to be noted however that at meals fluids are usually sipped,

TABLE 1

DOGS ANESTHETIZED WITH SODIUM BARBITAL BY STOMACH	TEMPERATURE	INITIAL LEUCOCYTE COUNT*	LEUCOCYTE COUNTS MADE AFTER TREATMENT (MINUTES)								REMARKS
			3	10	15	20	30	40	60	90	
	°C.										
	50	9,400	11,120		10,000		9,840				
	8	10,280	12,200		11,400		10,680				
	50	16,800	18,240		17,000		16,640				
I. 150 cc. water.	40	16,800	17,600	17,120			17,000				Distention only
Introduced	5	13,000	14,600	15,000		14,450	14,000				
rapidly by	40	13,120	14,520	13,480		13,000					Distention only
stomach tube	5	11,680	13,000	13,400		12,800	12,120	11,800			
	40	12,000	13,600	12,260		11,650					Distention only
	4	15,600	16,880	18,400		18,800	16,800	16,000	15,500		Slow return to
	50	15,260	17,200	16,680		16,000	15,750				basal level
II. Air 300 cc.		13,600	16,600	14,980		14,400	13,280				
Rapid dis-		13,120	15,200	14,000		13,420					
tention of		10,800	13,600	12,640		12,000	11,120				
stomach									Flow stopped		
III. Water (350 cc.)	40	10,000	9,860		9,600		9,820	9,500	9,800	9,460	Slight decrease
slowly intro-	40	10,120	9,580		9,280		9,600	9,480	9,780	10,000	Slight decrease
duced dur-	40	8,000	7,860		8,400		8,000	8,260	8,120		Slight decrease
ing 45 min-	55	7,600	7,920		8,000		8,180	8,300	7,980		No change
utes	55	12,800	13,000		12,860		12,920	12,780	12,700		No change

* Initial counts made about two hours after anesthetic was given. The high initial counts are abnormal but immaterial to this investigation.

not guzzled, that the reactions described are immediate and are recovered from within the usual meal period. Furthermore the reactions are independent of nutriment and therefore can have nothing in common with our usual concept of digestive leucocytosis, a condition which our experiences indicate to be wholly fictitious.

The failure of other experimenters to obtain similar results may be attributed to failure to recognize the necessity of eliminating all variables except the one under investigation and to compare all counts with a basal norm established by a condition of absolute rest.

SUMMARY

The low *basal* leucocyte count of the resting state is wholly unaffected by the intake of large quantities of either protein or carbohydrate.

Sudden distention of the human stomach, or abrupt changes in gastric temperature, due to hot or cold fluids, cause an immediate, but mild and transient rise in the leucocyte count. Animal experiments indicate that these reactions are due solely to reflex vascular disturbances, but have no relation to the absorption of digest.

All experiments indicate that there is no digestive leucocytosis in normal adults.

BIBLIOGRAPHY

(1) Molleschott, J. Wiener Med. Wochenschr. 1854.
(2) von Limbeck, R. R. Clinical pathology of blood. Tr. New Sydenham Soc., 1901.
(3) Arneth, J. Die Qualitative Blutlehre. Leipzig, 1920.
(4) Sabin, F., R. S. Cunningham, C. A. Doan and J. A. Kindwall. Bull. Johns Hopkins Hosp., 1925, xxxvii, 14.
(5) Shaw, A. F. B. Journ. Path. and Bact., 1927, xxx, 1.
(6) Smith, C. and A. M. McDowell. Arch. Int. Med., 1929, xliii, 68.
(7) Garrey, W. E. and V. Butler. Proc. Staff Meetings Mayo Clinic, 1929, iv, 157. Abstr. Internat. Congress Physiol., 1929, This Journal, xc, 355.
(8) Stetson, R. P. Arch. Int. Med., 1927, xl, 488.
(9) King, C. E., E. A. Blair and W. E. Garrey. This Journal, 1931, xcvii, 329.
(10) Walterhoefer, G. Deutsch. Arch. Klin. Med., 1926, cliii.
(11) Butler, V. and W. E. Garrey. This Journal, 1931, xcviii, 394.

THE EFFECT OF CAFFEINE ON THE PREVAGUS AND POST-VAGUS CHICK HEARTS

FLOYD J. BRINLEY

From the Zoölogy Laboratory, North Dakota State College, Fargo

Received for publication October 5, 1931

There seems to be a prevailing opinion among pharmacologists and physiologists that caffeine affects the force and frequency of the heart beat and that this effect is the result of the direct action of the drug on the cardiac muscle fibers. The work reported by Pickering (1) on Daphnia, Pitcher (2) and Heathcote (3) on mammalian hearts, indicates that dilute solutions of caffeine increase the rate and force of the heart beat while concentrated solutions decrease the frequency and amplitude of the beat. The general concept of the effect of caffeine on the cardiac muscle seems to have originated from the work of Pickering in 1893 (4). Pickering studied the effect of caffeine on the rate and rhythm of the cardiac contraction of 60 to 75 hour chick embryos. He was, therefore, studying the action of the drug on the heart before the entrance of nerves and apparently did not study the effect of caffeine on the innervated or neurogenic heart. Since the results of Pickering's experiments have been rather generally accepted without question and have remained unchallenged, it is deemed advisable to report his experiments, at this time, in some detail.

Pickering reported in his table on page 397, that dosages of caffeine equal to 0.00015 gram in one cubic centimeter of physiological saline solution (approximately equal to M/1400) increased the frequency of the heart rate from 88 to 100 per minute. Additional dosages of the same concentration applied to the same heart showed no further effect. More concentrated solutions, 0.0003 gram per cubic centimeter, reduced the rhythm of the same heart to 76 beats per minute: a dosage of 0.00075 gram proved fatal. In a second experiment, using a fresh heart, Pickering found that caffeine in a concentration equal to 0.0025 gram in a cubic centimeter of saline solution (approximately equal to M/100) produced a slight irregularity in the rhythm and the rate became much slower. He does not differentiate between the different chambers of the heart in these experiments, although he does make the following statement on page 384, "as I hope to show in the following pages that there are considerable differences in the attributes of the auricular contractile tissue and the ventricular tissue in the three day old chick embryo, it is important to bear in mind

that in my experiments the whole heart is under observation." The only place where Pickering mentioned the portion of the heart affected is in the last remark in the table where he states that "The auricular half of the heart gave two strong beats, the whole heart then passed into strong contraction and was irresponsive to stimuli." From these results Pickering concluded that caffeine affects the cardiac muscle directly and the nervous elements play only a secondary part. Since Pickering's experiments do not seem to be extensive enough to warrant his conclusions and as no experiments were conducted on the innervated heart, it was deemed advisable to study the effect of caffeine on the prevagus and postvagus hearts of chick embryos in order to ascertain whether the drug affects the rate and rhythm of the heart beat by its action on the cardiac muscles or on the nervous system.

METHOD AND PROCEDURE. Fertilized chick eggs (pure blooded white leghorns) were obtained from the Poultry Department of the State College and incubated at 37 degrees in an electric oven. The eggs were gathered from the hennery every hour and a half, therefore they were in about the same stage of development at the time of incubation. The embryos were allowed to develop for the desired period of time and an opening an inch in diameter was made in the shell and the eggs placed in an observation chamber where the hearts of the embryos could be observed. The observation chamber consisted of a small electric oven placed on its back so that the embryo could be observed through the glass door of the oven by the aid of a binocular microscope. The temperature of the observation chamber was maintained at the same degree as the incubator. After the embryos had recovered from exposure during the opening of the eggs and the heart rhythm became uniform, the time required for fifty cardiac contractions was taken with a stop watch. One-half of a cubic centimeter of caffeine (Merck U. S. P.) solution, made up in physiological salt solution and warmed to 37 degrees was placed in the opening in the egg shell directly above the embryo. In the earlier experiments the membranes surrounding the yolk of the egg were cut to allow caffeine to diffuse more quickly into the embryo but in later experiments this was found to be unnecessary and was discontinued. At various intervals after the addition of the drug the rate of heart beat was determined, as before, by taking the time for fifty cardiac contractions.

In the chick embryo there is no innervation of the heart for the first five or six days after incubation, therefore the heart beats without extrinsic nerves for the first 120 hours of incubation. After the vagus completes its connection with the heart, the frequency of the beat is presumably under the control of the nervous system. It is possible, therefore, to study the effect of caffeine upon the nerveless and innervated heart and in this way to determine if the drug affects the contractility, irritability or conduc-

tivity of the cardiac muscle or the frequency or amplitude of the beat by virtue of its action on the nervous system.

RESULTS. Eggs were incubated from 48 hours to ten days and the effect of caffeine solution on the embryonic heart was determined by noting whether or not any difference occurred after the addition of caffeine in the rate of contraction, force of heart beat or conductivity of the impulse. The addition of M/100 caffeine to the prevagus heart produced a decided irregularity in the auricular-ventricular rhythm. The auricles usually continue to beat at a regular and definite rate but the rhythm of the ventricular contraction is much slower. The ratio of the auricular to the ventricular beat may vary from 1–1 to 5–1. At other times the ventricular may beat at a reduced rate which is quite independent of the auricles. This condition approaches what is known as heart block in mammals. Gradually the entire heart is brought to a standstill in the diastolic condition. The cardiac muscle, however, still remains irritable and responds by contraction to mechanical and electrical stimulation.

The excised heart behaves in all respects the same as the intact heart in the embryo. The excised heart, after it has come to a standstill in caffeine solution, which may require an exposure of an hour or more, will remain irritable to electrical stimulation for several hours. If the excised heart is severed at the auricular-ventricular junction, the auricles will continue to beat for an hour or more while the ventricles soon come to rest in the diastolic phase. If the quiescent chambers are transferred to physiological saline solution, cardiac contractions are resumed within two or three minutes. Upon retransferring to the caffeinated solution the contractions soon cease.

The behavior of the prevagus heart treated with dilute solution of caffeine, M/1,000 or M/10,000 is different in some respects than when treated with stronger solution. The rate of the beat is slightly accelerated for a few seconds after the addition of the drug, followed by a gradual return to normal and after an exposure of several minutes the contractions of the ventricle become decidedly irregular while the auricles continue to beat at a normal and regular rate.

In the case of the older embryos there is a different situation. The embryonic heart is innervated by the vagus on the fifth or sixth day (Lillie). Therefore, in the older embryos the heart beat becomes somewhat complicated by the controlling action of the nervous system. The results obtained from the action of caffeine on the postvagus or innervated heart are different in degree from those described on the prevagus heart. Caffeine in the foregoing concentrations produce an initial stimulation which is quickly followed by an irregularity in the rhythm and a marked reduction in the rate of contractions of the entire heart. The frequency of the beat of the auricles as well as the ventricles becomes very slow with frequent

long pauses. During the period of inactivity the heart always remains in the diastolic condition and is sensitive to external stimulation. If the heart, stopped in caffeine solution, is removed from the embryo and kept in the same solution, the auricles will usually resume beating but ventricles seldom recover. If the excised heart is severed at the auricular-ventricular junction and the chambers placed in caffeine solution the auricles may continue to beat for two or three hours but the ventricles stop in diastole within a few minutes. They remain, however, irritable to external stimulation.

Conclusions. It is apparent from the foregoing results that the behavior of the prevagus heart is somewhat different than the postvagus when treated with caffeine solution. In the former condition the rate of the auricular beat is not significantly affected by the dilute solutions of the drug: the ventricles, however, show a decided irregularity and a marked reduction in the rate of pulsations. In the prevagus heart the beats are supposed to be the result of rhythmic contractions of the cardiac muscle fibers. The contraction wave originates in the sino-auricular node and is conducted through a system of modified muscle fibers, auricular-ventricular bundle, to the other chambers of the heart. If the conductivity of the fibers composing the auricular-ventricular bundle is decreased, the impulses arising in the sino-auricular node are completely or partially prevented from reaching the ventricles, therefore the latter chamber beats slowly or independent of the auricles. Any factor that influences the auricular-ventricular rhythm may be considered to affect the conductivity of the modified muscle fibers. This seems to be the case with caffeine.

In the case of the innervated heart the entire organ is more or less under the control of the nervous system and the entire heart shows the effect of the drug by an irregularity of the rhythm and a reduction in the rate, which is probably due to stimulation of the vagus nerve. It seems apparent, therefore, that the effect of caffeine may be twofold; 1, reduction in the conducting power of the muscles composing the auricular-ventricular bundle of His, and 2, stimulation of the vagus supplying the innervated heart. The drug does not seem to produce tetany in the cardiac muscle as is evident by the fact that the heart will respond by contracting after it has been brought to a standstill in caffeine solution.

SUMMARY

Prevagus and postvagus hearts of chick embryos were treated with M/100, M/1,000 and M/10,000 caffeine solution in order to determine if the drug affects the cardiac muscle or the nerves supplying the heart.

The results of a series of experiments indicate that caffeine in solution may destroy partially or completely the conducting power of the muscle fibers composing the auricular-ventricular bundle. It also depresses the rate of contraction of the innervated heart through its action on the vagus

nerves. Caffeine, in the concentrations used, does not produce tetany and apparently does not prevent contraction in the cardiac muscle.

BIBLIOGRAPHY

(1) Pickering, J. W. Journ. Physiol., 1894, xvii, 356.
(2) Pitcher, J. D. Journ. Pharm. Exper. Therap., 1912, iii, 609.
(3) Heathcote, R. H. Journ. Pharm. Exper. Therap., 1920, xvi, 329.
(4) Pickering, J. W. Journ. Physiol., 1893, xiv, 383.

STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

III. The Longitudinal and Circular Activity of the Distal Colon

HAMPDEN LAWSON[1] AND R. D. TEMPLETON

From the Physiological Laboratory of the University of Chicago, and the Department of Physiology of Loyola University School of Medicine

Received for publication February 8, 1932

In an earlier report (1) we described a type of motor activity characteristic of the distal colon of the dog, which was recorded by means of serial balloons as simultaneous pressure changes over long segments. This activity was most marked in the region of the internal anal sphincter, and was usually the only activity appearing in the distal colon. Occasionally the entire colon pulsated rhythmically throughout the course of long tracings, but the usual finding was anally-propagated activity in the proximal portion, with a reciprocal relationship to the rhythmic pulsations of the distal. It was assumed that the Pendelbewegungen described by the early German workers, and analyzed by Magnus (2) as longitudinal, might be responsible for such synchronous pressure changes over long segments. Other possible mechanisms which suggested themselves were rhythmic segmentation or haustral segmentation under the influence of a coördinating apparatus, or some muscular function peculiar to the colon.

This study is an attempt to separate the longitudinal from the circular activity of the distal colon, as a basis for further work on the nature of the rhythmic pulsations. It was necessary to select a method which would enable us separately to record changes in length and in the short diameter, and which could be used on unanesthetized dogs. An apparatus was constructed for this purpose (3), which consisted essentially of a movable plunger which could be inserted into a closed intestinal pouch, the plunger moving in a protecting tube to which tandem balloons were attached as previously described. Records were taken from water manometers of the pressure changes in the balloons. A similar pressure-volume record was obtained from movements of the plunger against a balloon, filled with air, and enclosed in a cylinder which was clamped outside the pouch.

The colon was transected in dogs which had previously been cecostomized for a balloon study of the intact colon. The left colic vessels were

[1] Donnelley Fellow in Physiology.

used as a landmark, the transection being made at different levels from 3 to 15 cm. above the point of entry of these vessels in the distal colon. The distal stump was invaginated and closed off to form a pouch opening by anus, and the proximal stump was sutured into the abdominal wound as a colostomy. There was considerable post-operative hemorrhage from the anus, and in one case complete prolapse of the pouch with subsequent death of the animal. In those dogs which survived the immediate effects of the operation, however, there was no significant loss of weight on the ordinary laboratory diet. One animal is still in use 13 months after operation.

The results reported below were obtained on unanesthetized animals, sufficient time, usually two weeks, being allowed for recovery from the operation. The dogs were trained to lie quietly on comfortable pads. The apparatus was inserted through the anus, and the plunger extended beyond its tube, against the end of the pouch, by inflating the balloon in the cylinder. The pressure in this balloon was usually about 10 cm. water with the apparatus adjusted and clamped in place. Inflation of the tandem balloons was carried out as in our previous work. Balloon tracings were also taken from the proximal segment at the same time with the distal record. In addition, six-balloon tracings were made of the entire colon, following transection, for comparison with records on the same dogs with intact colon.

In the following we have applied the term peristaltic, for convenience, to types of activity which appear on our balloon records as anally-delayed contractions (1).

Balloon tracings of the entire colon after transection in the region of the splenic flexure showed little alteration in the activity of either segment or in the reciprocal relationship between them (fig. 1). With the incidence of peristalsis in the proximal segment, the rhythmic pulsations of the distal segment were depressed. The rhythmic pulsations reappeared with the termination of the proximal type of activity, and often replaced peristalsis in the proximal segment. In only one dog, whose distal segment was so long as to include a portion of the proximal colon, did we observe spontaneous peristalsis in the distal segment (fig. 6). After transection, in the other animals, separation between proximal and distal types of activity occurred sharply at the site of transection. As in the intact colon, it was noted that a reciprocal relationship sometimes existed between distal activity and activity in the proximal colon which could not be classified from the record as peristaltic. In these tracings, however, many of the proximal periods of activity were unquestionably peristaltic. On the other hand, where only rhythmic pulsations appeared in the proximal as well as the distal colon, no proximal-distal reciprocity could be observed.

Records of the longitudinal and circular activity of the distal segment were found to vary with the length of the pouch, but were qualitatively

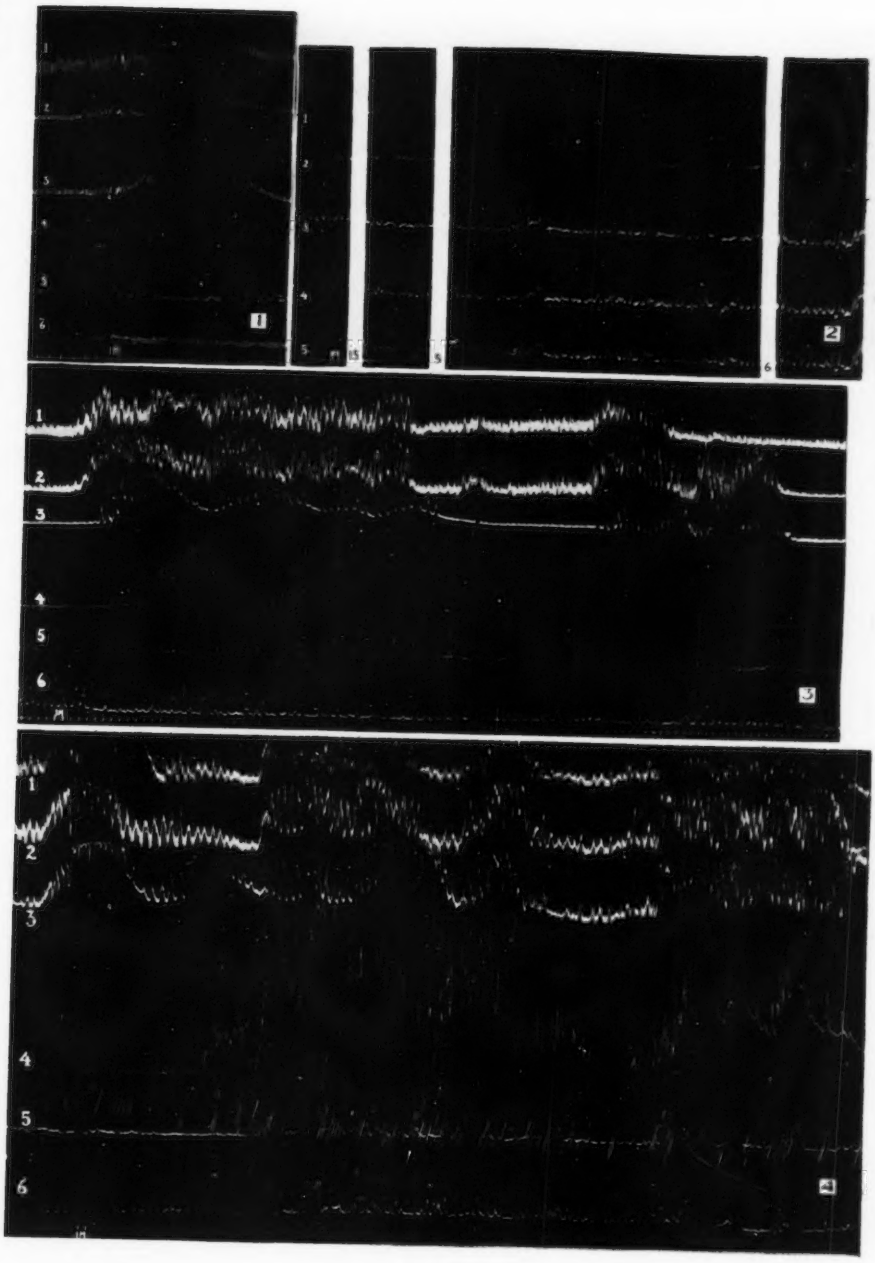
consistent in the dogs studied. The longitudinal tracings showed a marked periodicity, not unlike the periodicity noted in proximal balloon tracings. Unlike balloon tracings of the distal colon, longitudinal periods were frequently accompanied by high, lasting tone changes. The two types of contractions observed on balloon records were also seen on the longitudinal record, with similar rhythm. In most tracings, the uppermost balloon showed less activity than the lower, which was usually in the region of the internal anal sphincter (fig. 2). In many tracings only the plunger and the sphincter balloon showed activity, intervening segments remaining quiet. An invariable observation has been that the regular rhythmic type II activity of the balloons is depressed during activity of the plunger, sometimes becoming completely quiet (fig. 3), and sometimes, with high plunger activity, being replaced by an irregular type I activity on high tone (fig. 4), coincident with the peaks of the longitudinal tone changes. With cessation of plunger activity, there is recovery of the rhythmic type II contractions in the balloons. In one dog with a short distal segment (transection just above the left colic vessels) tracings usually showed no

Fig. 1. Six-balloon tracing of entire colon after transection just above left colic vessels. Balloons 1 to 3 are in proximal segment, 1 at level of ileo-colic junction. Distance between balloons approximately 5 cm. Balloons 4 to 6 are in distal segment, 6 at level of internal anal sphincter. Interval between balloons is approximately 3 cm. Time tracing in minutes.

Fig. 2. Distal colon activity as recorded by plunger-balloon apparatus. Transection 10 cm. above left colic vessels. 1 is manometer record of plunger movements. 2 is balloon at upper end of pouch, attached to extended end of plunger, and moving with the latter. 3 to 5 are fixed tandem balloons on plunger apparatus, 5 at level of internal anal sphincter. Interval between tandem balloons 3 cm. Time tracing in minutes. Rythmic pulsations progressively increased toward lower end of pouch, depressed during plunger activity except at peaks of plunger tone, when balloons show tone rise with irregular type I contractions. No evidence of peristalsis.

Fig. 3. 1 to 3 are tandem balloons in proximal segment. 4 is plunger, 5 and 6, balloons in distal segment. Colon transected 10 cm. above left colic vessels. Periods of plunger activity coincide with periods of peristalsis in proximal segment, and with depression of distal balloons. Distal balloons (5 and 6) show augmented activity during quiescence of plunger and proximal segment, except that during last period of proximal activity, appearing on balloons 2 and 3 only, not peristaltic, balloon 6 is augmented. Time tracing in minutes.

Fig. 4. Preparation and apparatus same as in figure 3. High plunger activity coincident with peristalsis in proximal segment. Distal balloons are not depressed during plunger activity, but the regular type II contractions on balloon 6 are replaced by irregular type I contractions on high tone during peaks of plunger tone changes. During high plunger tone the animal whined as if in pain, the tail was lifted, and the hind legs brought forward as in defecation. These movements usually coincided with the tone changes in the distal balloons, but not invariably. This figure was taken from a 6 hour tracing, in which this picture was periodically repeated. Time tracing in minutes.



Figs. 1 to 4

activity except in the plunger (fig. 5A). During longitudinal tone rises there was a slight depression of balloon tone, followed by a rise nearly simultaneous with the peak of the plunger tone change. Rarely, in this dog, the balloons also showed periods of activity, which occurred during the longitudinal period in such manner as to give the appearance of splitting the latter (fig. 5B). Following a period of general inactivity, two longitudinal periods would occur, with a brief intervening period in which the balloons alone were active.

The longest distal segment studied was one in which the transection was made about 15 cm. above the left colic vessels. In tracings from this

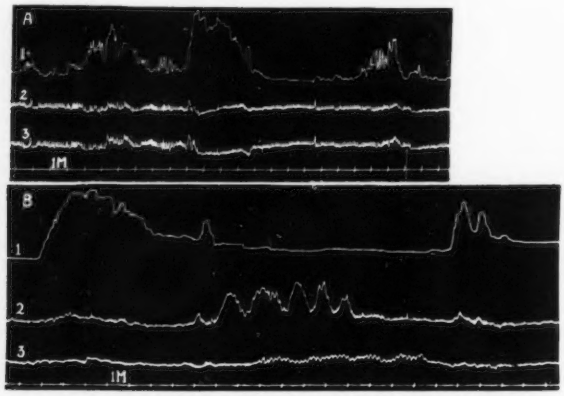


Fig. 5. Distal colon activity as recorded by plunger-balloon apparatus. Transection just above left colic vessels. 1 is manometer record of plunger movements. 2 and 3 are tandem balloons, 3 at level of internal anal sphincter. In A the plunger alone is active, balloon tone being slightly depressed during the rise of plunger tone. In B the balloons are also active, and periods of balloon activity bear a reciprocal relationship to periods of plunger activity. Time tracing in minutes.

animal, the appearance and relationships of the plunger record were quite similar to balloon records taken from the same dog's proximal colon before transection. Plunger activity occasionally preceded activity on the balloons below in such manner as to place the plunger tracing peristaltically into serial relation to the balloon records (fig. 6). When rhythmic pulsations were initiated, there was depression of the plunger.

The possibility, suggested in this observation, that movements of the plunger were produced by peristalsis sweeping the easily movable plunger periodically toward the anus, led to various modifications of the apparatus. The end of the plunger is capped with a metal ball. Reducing the size of this was found not to reduce either the amplitude or the rate of plunger

movements. Protecting the extended end of the plunger with a coil spring of such strength that, when the recording balloon was inflated against the plunger, the spring could be grasped tightly and compressed downward without moving the plunger, did not alter the plunger record.

In acute experiments (8) the belly was opened, the plunger apparatus inserted by anus, and the free end of the plunger stitched in place through the wall of the colon. These records show the same type of longitudinal activity as that recorded on unanesthetized dogs.

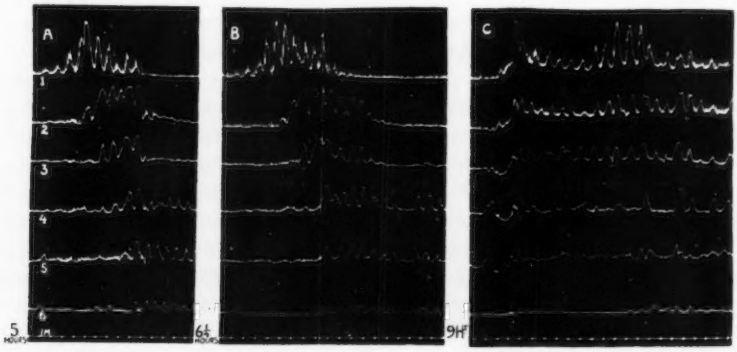


Fig. 6. Distal colon activity as recorded by plunger-balloon apparatus. Colon transected 15 cm. above left colic vessels. Representative periods of activity in a 12 hour tracing. Hours from beginning of tracing indicated between periods. Time tracing in minutes. 1 is manometer record of plunger movements. 2 to 6 are adjacent tandem balloons, 2 about 5 cm. below end of plunger, 6 at level of internal anal sphincter. In A plunger activity precedes and accompanies a period of peristalsis which can be traced as low as balloon 5. At this point rhythmic pulsations appear simultaneously on balloons 4 to 6, with depression of peristalsis and depression of plunger activity. In B peristalsis can be traced as low as balloon 4, at which time rhythmic pulsations are initiated throughout the segment, with depression of the plunger. Transition from peristalsis to rhythmic pulsations indicated by vertical line. In C there is an irregular, non-periodic activity, with failure of circular-longitudinal reciprocity.

As additional evidence that the plunger movements were not produced by the translational effects of peristalsis, we have obtained records from a small balloon tied on the end of the plunger, moving with the latter, and led out to a recording manometer by substituting for the plunger a small-bore metal tube with side-arm and flexible manometer connections projecting through the open portion of the plunger apparatus. Such tracings show typical plunger activity, without any evidence of peristalsis, the balloon at the extreme upper end of the pouch recording the same type of activity as is seen on the lower balloons, and following the other balloons in its periodic changes more closely than it follows the plunger (fig. 2).

Usually for a period of one hour or more following the insertion of the apparatus, the only activity recorded by the plunger is a gradual fall in tone. The balloons, during this period, are very active. At the time when the tone of the plunger tracing has fallen to a constant level, the balloon activity is usually much reduced, and it is at this time that periodic plunger activity appears. Assuming that the possibility of mechanical error in this portion of the tracings, such as inability to extend the plunger to the end of the pouch due to the demonstrably high circular tone and activity, might invalidate that portion of the record, we have discarded the first portion of each tracing in presenting these observations, and have continued the tracings until constant tone levels were obtained.

Balloon records from the proximal segment taken simultaneously with the records from the distal colon, showed, in general, that periods of plunger activity in the distal segment usually coincided with periods of proximal activity, if the latter were peristaltic. Usually the distal plunger tracing showed augmentation, and the distal balloons depression, slightly in advance of the proximal period (figs. 3, 4). The recovery of rhythmical distal balloon activity was associated with quiescence in the plunger, and either quiescence or rhythmic pulsations in the proximal segment. In one dog rhythmic pulsations were the only type of activity usually found in the proximal segment. We were unable, in tracings of this type, to establish any constant relationship between either the longitudinal or circular activity of the distal segment, and the proximal activity, although the longitudinal-circular reciprocity in the distal segment was constant. In tracings from the same animal, however, in which the more usual, peristaltic type of proximal activity was observed, longitudinal activity in the distal segment was more pronounced, distal longitudinal periods coincided with the proximal periods, and were associated with depression in distal balloon activity as described above (fig. 4). Often in all the animals studied, at the peaks of very high longitudinal tone changes, the tail would be lifted and the hind legs brought forward as in defecation. Occasionally the animal would whine as if in pain during these periods.

Using depression of proximal activity as an index (4) we compared the stimulating effect of the insertion and presence of the plunger apparatus, with that of the flexible balloon systems previously used. Such comparisons, made on two dogs, showed that in both cases the plunger apparatus produced longer and more complete depression than the flexible systems. In addition, it was noted that the insertion of the flexible systems into the distal pouch was followed by proximal depressions lasting as long as 75 minutes, as compared with a maximum of approximately 30 minutes in the intact colon. For some time after return of activity in the balloon nearest the cecum, the second and third proximal balloons were not fully recovered.

Learmonth and Markowitz in 1930 demonstrated the presence of inhibitor fibres to the distal colon of the dog in the lumbar colon nerves which reach the distal colon with the inferior mesenteric artery. (5) Our inability to find any gross alteration of the proximal segment after transection above this point might indicate that the distribution of such inhibitory fibres is largely distal to the cut. On the other hand, although the mesocolon was sectioned in our dogs to its root, we do not know what fibres from the inferior mesenteric ganglion reached the proximal colon through the root of the mesocolon. These workers stated, in addition, that motor fibres from the pelvic nerves ascended in the wall of the colon itself. We did not find a decrease in the activity of the proximal segment after transection.

Although the spontaneous motility of neither segment was altered after transection, our observations do indicate an increase in the irritability of the distal segment. Whether we are dealing here with an altered nervous control, or with some sort of adaptation following the transection and colostomy, our work does not show.

The reciprocity which we noted between typical proximal activity and typical distal activity was not diminished after transection. A purely mechanical basis, such as transfer of material, or the pull of one segment on another during activity, is thus excluded from the proximal-distal reciprocity, and the anatomical continuity of the nervous apparatus in the wall of the colon is shown not to be essential. The fact that Pearcy and Van Liere in 1926 (6) made similar observations in their study of the inhibiting effect of distention of any segment on the activity of other segments of the gastro-intestinal tract may be significant.

The assumption that a plunger such as we employed in the distal segment would not be moved by circular activity is probably unwarranted. New in 1899 (7) made this assumption in suggesting an "apparatus to show longitudinal movements of the intestine." His device consisted of two glass tubes, the one sliding within the other, producing pressure-volume changes on the contained air which were recorded by a tambour. In our apparatus we were able, by means of the tandem balloons, to determine whether or not circular activity appeared in the segment during movements of the plunger. In the isolated instance mentioned above in which balloon records showed peristalsis in the upper portion of the pouch accompanying plunger activity, the latter might be interpreted as simple translation toward the anus by the circular waves. This possibility is excluded, however, in all other cases by the absence of any such circular activity during plunger movements, and especially by finding in acute experiments (8) similar movements of the plunger during peristalsis, where translation is ruled out by sewing the end of the plunger into the gut wall. In our apparatus the size of the plunger was reduced to a minimum, about 2 mm.

in diameter of cross section. Reducing the size of the ball which capped the plunger, so as to afford less opportunity for waves of constriction to sweep it downward, and protecting the exposed end of the plunger with a spring whose coils would be moved by such waves, leaving the plunger unaffected, did not alter the record. It is doubtful if either of these modifications of the apparatus would completely protect the plunger from the influence of circular waves; it is significant, however, that they had no effect in reducing plunger movements.

A purely artificial basis for the circular-longitudinal reciprocity recorded by this apparatus is to be considered. It is possible that high circular activity in the region of the extended portion of the plunger might immobilize the latter, allowing plunger movements to occur only during circular inactivity. That this is not the explanation is shown by the fact that the upper end of the pouch is comparatively quiet, the circular activity being greater and greater as the sphincter region is approached. It is the latter region which reciprocates most strikingly with the plunger, a region in which circular activity could not affect movements of the plunger which here lies within its rigid protecting tube.

The relationship which we have recorded between the circular and the longitudinal activity of the dog's distal colon indicates a reciprocity which is not so invariable as would be predicted from the frequently quoted generalization of Exner (9): "that in a freely moving tube which contains as integral parts of its wall longitudinal and circular muscle, contraction of the former must bring about shortening of the tube and widening of its lumen, contraction of the latter lengthening of the tube and narrowing of its lumen." In 1883 Fellner (10) failed to confirm Exner's law in its entirety, on the distal colon of the dog, finding longitudinal relaxation frequently unaccompanied by circular contraction. He sectioned the dog's rectum, in acute experiments, attached the distal stump to a writing lever, and took simultaneous records from it and a balloon within the lumen. From his observations on the effects of nerve stimulation, he deduced a reciprocal innervation for the longitudinal and circular coats; the nervi erigentes supplying motor fibres to the longitudinal and inhibitory to the circular, the hypogastric motor to the circular and inhibitory to the longitudinal. Elliott and Smith, however, in 1904 (11) showed that stimulation of the pelvic visceral nerves in the dog had the same effect on both the longitudinal and the circular coat throughout the colon, shortening preceding constriction by about 30 seconds. Carlson (12) has reported that stimulation of the peripheral end of the sectioned hypogastric reverses the state of the distal colon, producing activity if the gut is quiet, and quiescence if it is active.

In the dog's distal colon we have seen the circular-longitudinal reci-

procity persisting for hours, only to be replaced by activity in which there is depression of the rhythmic circular type II activity during longitudinal periods, but no diminution in circular tone or type I contractions. This variability of the relationship between longitudinal and circular activity would indicate that the purely mechanical basis for such reciprocity suggested by Exner is not of prime importance in this region of the gut.

Luderlitz (13), Biedermann (14), Trendelenburg (15), and others have observed a longitudinal activity during peristalsis. Biedermann confirmed Exner's law in his work on the earthworm, stating that longitudinal contractions always preceded circular; Luderlitz saw shortening of the rabbit's small intestine as the first response to distention, with circular contractions much later; Trendelenburg found that during peristalsis there was a longitudinal contraction for every circular, preceding the circular contraction by about $\frac{1}{4}$ to $\frac{1}{2}$ wave length. Leusden and Riesser have recently criticized Trendelenburg's conclusions on the basis of their observation that the longitudinal-circular reciprocity could be altered at will by changing the pressure in the segment (16).

Our work indicates that there is a shortening of the distal colon during proximal peristalsis. Further, the shortenings of the distal segment stand in the same reciprocal relationship to the rhythmic pulsations of the distal colon as does proximal peristalsis. It is suggested that such longitudinal activity in the distal segment is the longitudinal component of proximal peristalsis, usually the only component which appears in the distal colon. In the intact colon we have reported a shifting point of separation between the proximal and the distal types of activity, peristalsis sometimes traversing the entire colon. After transection we observed the circular phase of peristalsis below the transection in only one dog, in which the transection was so high as to include most of the colon in the distal segment. In the other animals, with somewhat shorter distal segments, the transection, wherever it happened to be made, was the boundary between proximal and distal types of balloon activity. It would appear from these observations that either the transection itself, or the altered state of the empty distal colon after colostomy, interrupts the propagation of the circular phase of peristalsis more completely than that of the longitudinal phase.

Langley and Orbelli in 1911 (17) stated that the dragging down of the cloaca in the frog on stimulation of the sacral autonomic nerves was primarily due to contraction of special bands of muscle corresponding to the recto-coccygeal muscles of the mammal. We have not investigated the possible activity of such muscles in the dog. Observations of the perineum during periods of longitudinal activity have not given any indication that accessory muscles were active unless defecation movements were obtained. Our longitudinal tracings are strikingly similar to those presented by

Fellner (10) in which curare was given in amounts sufficient to paralyze the respiratory muscles, and in which he assumed that the activity of such muscles was ruled out.

The stationary contractions of the dog's distal colon which we have termed rhythmic pulsations are shown by these observations to be due not to contractions of the longitudinal coat, which presumably would present the picture of synchrony over long segments, but to contractions of the circular coat unaccompanied by generalized shortening of the active segment.

The writers wish to acknowledge their indebtedness to Dr. A. J. Carlson, under whose direction this work was conducted.

SUMMARY

1. Transection of the dog's colon in the region of the splenic flexure does not produce marked alteration in the spontaneous motor activity of either segment.

2. The reciprocal relationship observed in the intact colon between the proximal and distal types of activity is preserved after transection.

3. Peristalsis as a wave of constriction does not pass from the proximal to the distal segment over a transection in the region of the splenic flexure.

4. The longitudinal activity of the distal colon bears a reciprocal relationship to the circular activity of that segment, the relationship being most marked in the anal sphincter region, which shows the greatest circular activity.

5. The longitudinal activity of the distal colon runs parallel to proximal peristalsis, if the latter is present in the proximal segment. With other types of activity in the proximal segment, the distal longitudinal activity shows no constant relationship.

6. The irritability of the distal colon is increased after transection and colostomy.

7. The rhythmic pulsations of the distal colon are constrictions unaccompanied by generalized shortening of the segment.

BIBLIOGRAPHY

(1) Templeton and Lawson. 1931. This Journal, xcvi, 667.
(2) Magnus. 1904. Pflüger's Arch., cii, 123.
(3) Templeton and Lawson. 1931. Journ. Lab. Clin. Med. (in press).
(4) Lawson and Templeton. 1931. This Journal, xcix, 87.
(5) Learmonth and Markowitz. 1930. This Journal, xciv, 501.
(6) Pearcy and Van Liere. 1926. This Journal, lxxviii, 64.
(7) New. 1899. Proc. Physiol. Soc., xxiv, 26.
(8) Templeton and Lawson. Unpublished data.
(9) Exner. 1884. Pflüger's Arch., xxxiv, 310.

(10) FELLNER. 1883. Med. Jahrb. d. Ges. d. Aerzte, 571.
(11) ELLIOTT AND SMITH. 1904. Journ. Physiol., xxxi, 272.
(12) CARLSON. 1930. Journ. Amer. Med. Assoc., xciv, 78.
(13) LUDERLITZ. 1889. Virchow's Arch., cxviii, 19.
(14) BIEDERMANN. 1904. Pflüger's Arch., cii, 475.
(15) TRENDELENBURG. 1917. Arch. f. exp. Path. u. Pharm., lxxxi, 55.
(16) LEUSDEN AND RIESSER. Arch. f. exp. Path. u. Pharm., cxx, 77.
(17) LANGLEY AND ORBELLI. 1911. Journ. Physiol., xli, 450.

AN ANALYSIS OF THE RHYTHMIC VASCULAR CHANGES IN THE UTERUS OF THE RABBIT

J. E. MARKEE

From the Department of Anatomy, Stanford University[1]

Received for publication February 2, 1932

A number of the characteristics of the rhythmic vascular changes in the uterus of the rabbit have already been described (Markee, 1929, 1932). This study deals with the factors that determine the duration of the vascular cycle, the phases of the vascular cycle, the effect of adrenalin on the cycle and the relation of the vascular to the muscular cycle.

I. FACTORS INFLUENCING THE LENGTH OF THE CYCLE. Since different transplants in the same eye have independent rhythms it seemed advisable to consider more fully the factors that might influence the duration of the cycle.

A. *Part of the uterus from which the transplants are taken.* In the intact uterine horn, the vasoconstrictions begin at the tubal end and pass down toward the cervix. A few seconds later vaso-dilatation begins at the cervical end of the uterine horn and passes towards the tube. This suggests that the tubal end may be a regulator or "pacemaker" for the vaso-constrictions. If the conditions in the endometrium are similar to those in cardiac musculature, one should expect transplants of endometrium from the tubal to have a shorter cycle than those from the cervical region if a regulator exists. Double transplants were hence made to the eyes of 12 rabbits, each receiving one piece from each end of the uterine horn but the difference in the duration of the cycles of the paired transplants from opposite ends was neither constant enough nor great enough to be decisive.

B. *Microscopic structure of the transplants.* Although the microscopic structure of the transplants is very similar to the structure of the endometrium in its normal position, there was a slight increase in the amount of fibrous connective tissue in all transplants examined. In general, those transplants that have the most noticeable increase in the amount of fibrous connective tissue have the longest cycles. It therefore seems probable that the increase in fibrous connective tissue may be correlated with the lengthening of the rhythm.

The arrangement of the blood vessels in the transplants has been studied

[1] Aided by a grant from the National Research Council Committee on Research in Problems of Sex.

by the injection method and by the examination of thick sections of uninjected material. There does not seem to be any significant alteration in the vascular patterns in endometrium transplanted to the iris. It was previously shown that some, if not all, of the functional vessels in the transplant were present in the uterus before implantation. However, it seems that the architecture of the vascular pattern may be altered somewhat when the iridial vessels anastomose with the cut ends of those in the transplant, and that this slight modification in the pattern may be another factor in the determination of the duration of the vascular cycle. There is considerable variation in the amount of time that elapses between transplantation and the re-establishment of circulation within the transplants. Those transplants that vascularize most rapidly have the shortest cycles and the greatest number of blood vessels per unit area, as established by observation in the living animal and by examination of stained sections. Although these three factors have been considered separately it is evident that their occurrence and their effect on the vascular rhythm are intimately related.

Since Hofbauer (1929) described myoneural bundles in the human uterus, it seemed possible that they are the ones that control the vascular rhythm. These elements, if present in the guinea pig and in the rabbit, are not grouped together in a well defined bundle as they are in man and it was not possible to show that they are causally related to the vascular rhythm either in the endometrium *in situ* or in the transplants.

C. *The local use of nicotine.* Since the wave of the vasoconstrictions passed down the uterus towards the cervix and the vasodilatation in the opposite direction, it seemed feasible to apply nicotine locally to prevent the passage of any nervous stimuli along the uterine horn. When a solution of nicotine is painted on the uterine horn near the middle the vasoconstrictions pass down to this area but not beyond it and none occur below it but the results were far from satisfactory because after a few minutes the vascular changes ceased. The vasodilatations seem to be the passive part of the vascular cycle for they will originate where the uterus is bathed in nicotine. The vaso-constrictions, on the contrary, will neither pass that point nor originate below it.

D. *Transection of a uterine horn.* In view of the fact that the vasoconstrictions would not pass the area painted with nicotine it seemed advisable to discover whether the upper and lower portions of the uterine horns had a common rhythm when divided from each other. Therefore, the uterine horn on one side was transected about half way between its two ends. Since such a trauma arrests the vascular changes for some time, the animals were examined three days later. At that time there was only a slight difference in the rhythm in the two parts, that in the upper segment being slightly shorter than that in the lower. The rhythm on the unoperated

side was slightly shorter than that in the upper segment on the operated side. Therefore, there is very little evidence in support of the hypothesis that a regulator exists in the tubal portion of the uterine horn.

II. The phases in the vascular rhythm. In addition to being modified by the time of the day and the amount of the follicular hormone present in the blood, the vascular cycle is also modified by fright. If a rabbit is frightened during the middle third of a vascular cycle, a vasoconstriction occurs 17 to 20 seconds later. Fright in any other part of the cycle does not modify either that or a subsequent cycle. Therefore, each vascular cycle may be divided into four periods or phases: a period of vasoconstriction (5 to 15 seconds); a refractory period (20 to 35 seconds); a resting period (10 to 30 seconds); and a latent period (17 to 20 seconds).

Fright was followed by vasoconstriction only during the resting phase. Since this period is more variable in length than the others, it largely determines the length of the individual cycles. Thus during the hours of the day when the cycles are longest, this period is greatly lengthened, and it is shortened proportionately during the hours of the day when the cycles are shortest. The period of vasoconstriction is the only other one the length of which is variable. By refractory period is meant the time during which vasoconstriction cannot be induced by fright. This period is constant in any given transplant but differs in length in different transplants and so do all the others. The latent period is the interval between the time that the animal is frightened and vasoconstriction.

A. *Multiple transplants.* That the "phase or period mechanism" lies within, rather than without, the uterus is indicated by the reactions of different transplants in the same eye to fright. Since different transplants to the same eye have independent rhythms, it is possible to frighten an animal when one transplant is in the resting and another in the refractory period. If this be done, vasoconstriction occurs only in the transplant that is in the resting period.

B. *Removal of the adrenals.* The latent period of 17 seconds is so long that it hardly seems that the stimulus for vasoconstriction can be a nervous reflex. Moreover, it has already been shown that stimulation of the cervical sympathetic does not modify the vascular cycle. In order to discover the effect of adrenalectomy on it, both of the adrenals were removed from 12 female rabbits having successful transplants.

It was not possible to induce vasoconstrictions in the transplants of these animals either immediately after the operation or later by frightening them. Only the rhythmic vascular changes persisted and the cycles were lengthened as the animals weakened.

C. *The latent period.* Stuart and Rogoff (1916) found that the time required for the blood to pass to the eye from the adrenals was several seconds less than the latent period of mydriasis produced by splanchnic stimulation.

They injected 1.5 to 2 cc. of a 1/50,000 solution of adrenalin with methlyn blue into the femoral vein and noted that mydriasis is nearly equal to the interval between the injection of adrenalin into the femoral vein and mydriasis. Although stimulation of the splanchnics and frightening the animal are very different, the above facts may, with a fair degree of safety, be regarded as indicating that adrenalin reaches the blood stream very soon after an animal is frightened. If that assumption is valid, only a very small part of the latent period of 17 to 20 seconds is accounted for by the time required for adrenalin to reach the blood stream after fright. Koppanyi (1930) found that the circulation time in the rabbit was about 6 seconds, and that the pulmonary circulation time was a little less than half of that. It therefore seems that 13 to 17 seconds elapse between the time that adrenalin reaches the eye and the appearance of vasoconstrictions in the uterine transplants, from fright.

Various amounts of adrenalin were injected intravenously (see table) and the latent period, the duration of the vasoconstrictions and the time of

TABLE 1

Effect of injection of adrenalin on blood vessels in uterine transplants (18 rabbits)

MGM. PER KILO	LATENT PERIOD	PERSISTENCE OF VASOCONSTRICTION	REAPPEARANCE OF CYCLE
0.00002 mgm.	17 seconds	8 seconds	75 seconds
0.0006 mgm.	17 seconds	14 seconds	75 seconds
0.001 mgm.	17 seconds	25 seconds	180 seconds
0.005 mgm.	9 seconds	140 seconds	12 minutes
0.01 mgm.	6 seconds	252 seconds	22 minutes

reappearance of the vascular cycle were noted. The length of time that the vasoconstrictions persisted increased as the amount of adrenalin injected was increased. However, the increase in the length of time that the vasoconstrictions lasted was not proportional when large amounts of adrenalin were injected. The length of time that the vasodilatation persisted was also roughly proportional to the length of the period of vasoconstriction. The ratio was about 6 to 1 as it is in the spontaneous vascular cycle. When adrenalin was injected slowly the effect on the vascular rhythm was proportional, not to the total amount injected, but to the amount injected within the first 15 seconds. It therefore seems that either in the normal animal the vessels of the uterus react only to the adrenalin that is liberated during the first 15 seconds after the animal is frightened or that the adrenals only secrete adrenalin for 15 seconds. Since fright may be very prolonged, the first alternative must be true and as a result this method can be of value only in a study of the amount of adrenalin produced during the first 15 seconds of fright.

If a rabbit is merely slightly startled during the resting period the subsequent vasoconstriction persists about 8 seconds, but if it is severely frightened the vasoconstriction persists for about 25 seconds. Since the injection of 0.0002 mgm. per kgm. causes a vasoconstriction that persists for 8 seconds and 0.001 mgm. a vasoconstriction that persists 25 seconds, these amounts may represent the range of secretion of adrenalin during the first 15 seconds of fright in the rabbit. The fact that the latent period of the injection of small amounts of adrenalin is the same as the latent period after frightening an animal also suggests that adrenalin reaches the blood stream almost immediately after a rabbit is frightened.

Injections of adrenalin were made in the different periods of the vascular cycle. It was found that the smaller amounts (0.0002 to 0.001 mgm.) would not induce a vasoconstriction if injected in the refractory period. However, the larger amounts (0.0005 to 0.01 mgm.) induced a vasoconstriction in any period of the vascular cycle. It therefore seems that amounts 5 times greater than those normally produced will "break through" the refractory period.

Somewhat similar results were obtained by making one injection and following that with another. If 0.0002 mgm. is injected, a vasoconstriction occurs and the uterine vessels are then refractory for 40 seconds to another injection of 0.0002 to 0.0006 mgm. of adrenalin. However, they are not refractory to 0.005 mgm. or more. After relatively large injections the refractory period is lengthened, for further injection of from 0.0002 to 0.0006 mgm. does not induce a vasoconstriction 5 minutes after the injection of 0.01 mgm. However, slightly different results were obtained by following one relatively large injection by a second injection of the same amount. Although the injection of 0.01 mgm. was followed by a vasoconstriction that lasted over 4 minutes, and this by a vasodilatation lasting 22 minutes, a second injection of the same amount within 5 minutes induced a vasoconstriction which lasted only $2\frac{1}{2}$ minutes. It appears that small injections are followed by a refractory period and the larger by a period of fatigue or at least a period of lessened response. That the refractory period after the injection of small amounts is not merely a period of fatigue is shown by the fact that a second injection at the end of the refractory period induces as long a period of vasoconstriction as the first injection did, while the injection of a large amount of adrenalin even 10 minutes after a previous injection induces a shorter period of vasoconstriction.

There are two possible explanations of the vasoconstrictions that follow the injection of the smaller amounts of adrenalin. They were induced either by the adrenalin injected or frightening the animal at the time of injection. Four facts indicate that the first was the case. The injection of saline did not induce a vasoconstriction; injection while one transplant was in a refractory period and another in the resting period induced a vasocon-

striction only in the latter; the length of the vasoconstriction was proportional to the amount of adrenalin injected; and they were temporarily related, not to the insertion of the needle into the vein, but to the actual injection of the adrenalin.

The latent period is shortened as the amount of adrenalin injected is increased. When 0.01 mgm. is injected the latent time is equal to that found by Koppanyi (1930) in the iridial muscles, that is, 3 seconds. However, when from 0.0002 to 0.001 mgm. are injected the latent time is 14 seconds (allowing of course 3 seconds for the adrenalin to reach the eye).

Transplants that ruptured the eyeball. A single large transplant of a portion of the uterus was made to the eye of a 31 day old rabbit. The uterine horn at that age is so thin that some muscle was unavoidably included in the transplant which was so large that it nearly filled the anterior chamber of the eye. When the animal became sexually mature there was a marked bulging of the eye because of the growth of the transplant at this time. The animal was mated with a vasectomized male. About 38 hours later the eye ruptured and a portion of the transplant about 1.7 cm. long, and 0.8 cm. wide, 0.6 cm. thick, protruded through the lesion. Since this mass filled the opening there was very little escape of aqueous and this ceased after 3 hours. Since the extruded mass showed no signs of becoming infected and the animal did not seem to be in pain, it was decided to watch the transplant closely. Eight days later it began to decrease in size and continued to do so until 19 days after mating. This suggests that the growth and regression of the transplant was under the control of the secretion of the corpus luteum.

Since the extraocular portion of the transplant offered unusual possibilities for observation, the vascular cycle and the contractions of the uterine muscle as judged by movement of the mass were observed. After the animal was killed, the transplant was sectioned serially, stained, and examined microscopically for any changes in structure that might have been caused by changes in temperature, dehydration or trauma.

Vascular rhythm. The rhythmic vascular changes in the transplant were interrupted in vasodilatation, 7 hours after the animal had been mated and did not reappear until 10 days after mating. They had returned to their normal rhythm 13 days after mating and remained normal through the rest of the period of observation. A spontaneous cycle 65 seconds long persisted and in addition the typical vasoconstrictions of fright were observed.

Muscular contractions. When the mass protruding from the eyeball was relaxed, it hung down so that the lower portion of it was in contact with the cornea for about 1 cm. below the rupture point. When it contracted it seemed to shorten and become broader and the dependent portion seemed to be lifted forward so that it was separated from the cornea by a space from 0.1 to 0.2 cm. wide. The contractions observed were of two kinds, the rhythmic and the fright contractions.

Rhythmic contractions. As might be anticipated from Reynolds' (1930) work on uterine fistulae, no muscular contractions were observed in the uterine transplant for the first 12 days after mating. From that time on movements in the muscle mass were observed about every 110 seconds. It seems worthy of note that the muscular cycle was nearly twice as long as the vascular one and that it was more irregular temporally. Since the two cycles were of unequal length, it was impossible for them to occur simultaneously except at intervals.

Fright contractions. When the animal was frightened, the muscular contraction sometimes occurred 17 seconds later. The length of the latent period and the uncertainty of the response suggested that there might be periods in the muscular cycle similar to those in the vascular one. A contraction could be induced only during the resting phase and frightening the animal in any other phase had no effect on the muscular cycle. The contraction period lasted about 10 seconds, the refractory period about 30 seconds, and the resting period about 50 seconds, and the latent period 17 seconds.

Since the muscular and vascular cycles were of different lengths, frightening the animal had the four following results. Fright induced during the refractory period of both cycles induced neither vasoconstrictions nor muscle contraction. Fright during the refractory period of the vascular cycle and the resting period of the muscular cycle was followed only by contraction of the muscle. Fright during the resting period of the vascular cycle was followed by vasoconstriction, and fright during the resting period of both cycles was followed by simultaneous vasoconstriction and muscle contraction. The length of the contraction of the muscle was also roughly proportional to the degree of fright induced, lasting about 8 seconds after the animal was merely startled and as long as 25 seconds when the animal was severely frightened. Unfortunately it was impossible to ascertain by this method whether or not the intensity of the contraction was increased by frightening the animal more severely. After either the constrictions or the muscular contractions had begun, prolonging the period of fright did not prolong the response.

Injections of adrenalin (0.0002 to 0.0006 mgm.) cause a muscular contraction if the adrenalin reaches the eye during the resting period of the muscular cycle. If larger amounts are injected (from 0.005 to 0.01 mgm.) muscular contraction is induced in any period of the muscular cycle.

Reynolds states that the presence of the hypograstric nerve may account for the rhythmic muscular changes that he has recorded. In view of the fact that the rhythmic muscular contractions in the transplants have the same rhythm as those that he has recorded, the rôle of the hypogastric nerve seems to be excluded. Further, no nerves were found growing into the transplant, a fact which might have been anticipated since Raul (1930)

found no nerves growing into or out of transplants of fetal nervous tissue in the anterior chamber of the eye. Rhythmic contractility seems to be an inherent characteristic of uterine musculature and the vascular and muscular cycle is similar in several respects.

DISCUSSION. The presence of a gradient in the rhythmic vascular changes in the uterus of the rabbit brings up the question as to whether or not the blood supply is the significant factor and also suggests that there may be loosely arranged myoneural elements in the uterus of the rabbit similar to the ones described in the human uterus by Hofbauer.

The lengthening of the resting period during the time of day when the vascular cycles are longest seems to indicate that the variations in the cycle found both in this species and in the guinea pig during different parts of the day may be explained on that basis.

Since the vasoconstrictions that follow frightening the animal are not present after the adrenals have been removed it seems that these vasoconstrictions may be caused by the elaboration of adrenalin when an animal is frightened. Since contractions of the uterine muscle follow fright, the question arises as to what effect fright might have on the uterus during pregnancy and especially near the end of gestation, and what relation vasoconstrictions and uterine muscular contractions may have to the subjective experiences that woman undergoes when frightened.

Since rhythmic contractions of the uterine muscle occurred in transplants without any nerve supply, it would seem that these are an inherent characteristic of uterine muscle. Since they were not present during the period of pseudo-pregnancy, they may be arrested by one of the hormones that is present in increased amounts at that time.

This investigation indicated that from 0.0002 to 0.001 mgm. of adrenalin may be liberated per kilogram of body weight in the rabbit during 15 seconds of fright, but this method does not seem suitable to determine how much may be liberated during longer periods of fright. Cannon presented evidence that indicates that adrenalin is not continuously secreted by the resting animal. The results obtained in the present study may therefore represent the total amount of adrenalin that is secreted during fright rather than merely the increased amount that is secreted when the rabbit is frightened.

Cori (1931) has presented evidence that 0.0005 mgm. per minute of adrenalin will induce hyperglycemia in the rabbit. The results of the present study hence suggest that when a rabbit is merely startled, 16 times as much adrenalin is secreted per minute as is necessary to induce hyperglycemia and that in severe fright 48 times the amount of adrenalin required to induce hyperglycemia is liberated per minute. It also appears that from 3 to 12 times the amount required to produce glycosuria is liberated (Trendelenberg, 1923).

It is not definitely known that vasoconstriction or muscular constriction for longer than 25 seconds would be injurious to the uterus. However, the presence of a mechanism that prevents their continuation beyond 25 seconds suggests that they might be and that the utility of this mechanism and of the refractory period may be the prevention of injury to the uterus when fright is very prolonged.

SUMMARY

The uterine vascular cycle may, for the sake of clarity, be divided into 4 periods; the period of vasoconstriction, refractory period, resting period, and latent period.

The uterine muscular cycle may be divided into similar periods.

In a transplant of uterus in the anterior chamber of the eye of a rabbit, the two cycles may be independent.

Frightening an animal causes a contraction or a vasoconstriction only during the resting period.

The injections of from 0.0002 to 0.001 mgm. of adrenalin induced vascular changes in the uterine transplants similar to those that occur 17 seconds after a rabbit is frightened.

The technique of intraocular implantation appears to have been originated by J. Cohnheim (1877) and was used by Salomonsen (1879), Baumgarten (1880), and Klebs (1883). However, I believe that Schochet was not aware of this and I hence desire to acknowledge my indebtedness to him for the technique of implantation.

BIBLIOGRAPHY

Baumgarten. 1880. Berl. klin. Wochenschr., nos. 49, 50.
Cannon, W. B. 1931. This Journal, xcviii, 447.
Cohnheim, J. 1877. July 13. Stzgsb. d. Schles. Gesellsch. f. vaterland. Cultur.
Cori, C. F. 1931. Physiol. Rev., xi, 143.
Hofbauer, J. I. 1929. Journ. Amer. Med. Assoc., xcii, 540.
Klebs, E. 1883. Arch. f. exper. Path. u. Pharm., xvii, 1.
Koppanyi, T. and A. Liverson. 1930. This Journal, xciv, 564.
Markee, J. E. 1929. Amer. Journ. Obst. and Gynec., xvii, 205.
1932. This Journal, c, 32.
Raul, M. M. 1930. Arch. d'Anat. Microscop., xxvi, 3.
Reynolds, S. M. and M. H. Friedman. 1930. This Journal, xciv, 696.
Salomonsen. 1879. Nord. med. Arkiv., 11.
Schochet, S. S. 1920. Surg., Gyn. and Obst., xxxi, 148.
Steuart, G. N. and J. M. Rogoff. 1916. Journ. Pharm. Exper. Therap., iii, 205.
Trendelenberg, P. 1923. Pflüger's Arch., cci, 39.

CARDIOVASCULAR RESPONSE OF HEALTHY YOUNG MEN TO POSTURAL VARIATIONS AT VARIED TEMPERATURES[1]

JOSEPH E. HAMILTON, JOSEPH S. LICHTY AND WILLIAM R. PITTS

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication December 30, 1931

In this study we report the circulatory reactions of young men subjected to rather drastic conditions. Prolonged standing without even slight motion and without increase in breathing frequently results in fainting. The position imposes a definite burden upon the circulatory apparatus. Turner (1927a, 1927b, 1929) carried out a series of experiments on the circulatory response of college girls during prolonged quiet standing. She measured systolic, diastolic, and pulse pressures and the pulse rate. On the basis of these records she developed a system of grading circulatory efficiency which will be referred to as the "standing test." It was suggested that if standing could be divested of all muscular effort, the effects of gravity on the circulation might become even more definite and, further, if during this "passive standing" the subjects were subjected to high temperatures, their power to compensate might become still less. These expectations have not been realized, and our hope that we might be able to gather data helpful in determining normal circulatory fitness and ability to withstand high temperatures has not been realized.

METHODS. We have employed a tilting table which makes it possible to maintain the subject entirely passive while being moved from horizontal to vertical. The apparatus consisted of a padded table balanced on a horizontal axle. By means of an electric motor the table could be slowly revolved from the horizontal to the vertical position. This 90° excursion required about 18 minutes. A pointer indicated the varying angle on the dial bolted to the frame. An adjustable foot board was fitted to the subject's length, so that when his feet were resting flatly upon it, his shoulders were pressed snugly into the padded shoulder braces. The subject's knees were slightly flexed over a pillow placed on the table beneath them. Two wide, web straps were secured across the legs, one just below and the other just above the knees. A third strap was passed across the body at the level of the symphysis pubis. These arrangements allowed a maximum support to the body without compressing the abdomen. The heart rate and the systolic and diastolic pressures were recorded at every 10°, beginning at 0°. During the experiment the subject remained absolutely quiet and as relaxed as possible. The experiments were carried out in a room

[1] The expenses of this investigation were met in part by a grant from the DeLamar Mobile Research Fund.

which was automatically maintained at any desired temperature. The absolute humidity at the four different temperatures used remained constant, since no moisture was introduced during the process of heating. A table somewhat similar, but without a motor to provide constant motion, was used by Turner, Newton, and Haynes (1930) as part of a series of experiments, the primary purpose of which was to demonstrate a slowly lessening return of blood to the right side of the heart as the subject slowly became upright.

EXPERIMENTAL OBSERVATIONS. The subjects were medical students, ranging between the ages of 20 and 27 years. Just before each experiment, each man was given the Turner "standing test" in order to correlate and compare the tilting table response with that in the standing test. After the "standing test" the subject reclined for from 10 to 15 minutes until his blood pressures and pulse rate were again constant. He was then secured comfortably to the table in the horizontal position, and again pulse and blood pressure readings were taken until they became steady. These were the "0°" readings. The motor was then started, and the subject was revolved through 90° to a standing position. Without stopping the motor, the pulse rate and blood pressure readings were taken at every 10°, from 0° to 90°. After the subject reached the vertical, he was promptly returned to the horizontal level by hand, and a second 0° reading was taken. Three other readings were taken at 0° at intervals of 2, 4, and 6 minutes, respectively, after the return. Eleven experiments were carried out at room temperature, 19 at approximately 90°F., 24 at approximately 120°F., and 21 at approximately 130°F. The total number of subjects was 21; no subject was used more than four times at any one temperature. Eight of the subjects, for whom there were readings at each of the four temperatures, were placed in a group for special study, the idea being that if the temperature groups were composed of the same subjects at each temperature, the effect of the different temperatures would be brought out better than by a general group, in which some of the subjects would appear at one temperature and some at another. The results from this special group, however, were so similar to those of the general group that they have been omitted as unnecessary.

The data are presented in graphic and tabular form. Figure 1 consists of four graphs made from the composite responses of all subjects. The graphs for all four temperatures show certain general characteristics.

1. The systolic blood pressure remains nearly level throughout, with perhaps a slight rise up to 50° or 60° and a slight fall thereafter.

2. The diastolic blood pressure approaches the systolic curve up to 80°, and then declines from 1 to 2 mm. or remains level until 90°.

3. The resulting pulse pressure shows a steady diminution as 90° is approached.

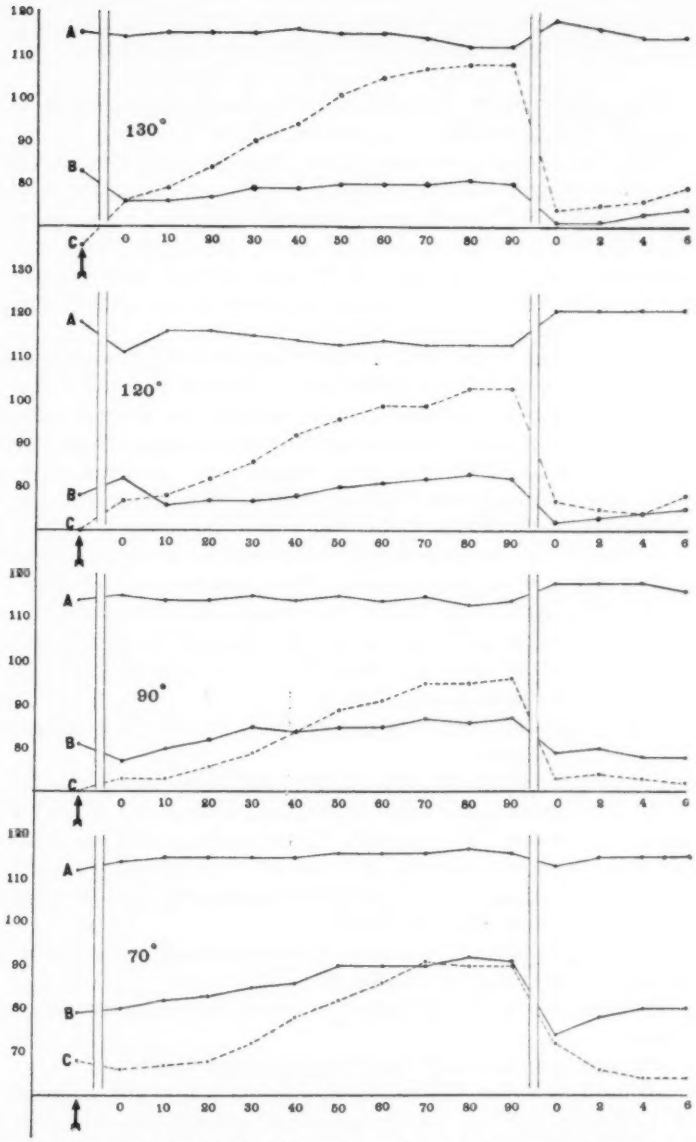


Fig. 1. Composite graphs showing pulse and pressure responses to posture at 70°, 90°, 120°, and 130°F. *Abscissae*, degrees of tilting. The arrow indicates "control" or reclining at room temperature; 0 to 90 are the angles of tilting from horizontal to vertical; 0 to 6 indicate the return to horizontal and 2, 4 and 6 minutes thereafter. *Ordinates*, both millimeters of mercury of pressure and beats per minute of pulse. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate. *Double lines*, breaks in continuity in the three curves *a*, as the subject moved from reclining outside to reclining inside the constant temperature room, and *b*, as the subject returned to horizontal from vertical.

4. The pulse response, far more sensitive than the pressures to changing posture, mounts steadily to a maximum at 80° or 90°.

5. When the subject is returned to the horizontal, the constant effect is a prompt widening of the pulse pressure in excess of that at the beginning of the experiment. This widening is produced by both a rise in the systolic pressure and a fall in the diastolic pressure. The pulse drops promptly to practically its initial reading. Pulse and pressure have not quite returned to initial readings at the end of 6 minutes, a time arbitrarily chosen to show the trend of the return to initial readings.

It was surprising to find such a slight change in response at the higher temperatures. Aside from a preliminary adjustment of pulse rate and pulse pressure between reclining at room temperature (control) and reclining in the heat (0°), the curves described by these readings at high temperature are very similar to those at room temperature. In short, the cardiovascular system is easily able to adjust itself to these higher temperatures. The subjects, aside from the discomfort in the heat, seemed on the whole to show little, if any, more tendency to faintness at the high temperatures than they did at room temperature. The procedure was stopped three times at 120°F. and once at 130°F. due to faintness of the subject. Two of these faints, one at 120° and one at 130°, occurred in the same subject, and all four occurred above the angle of 50°. All subjects perspired profusely, but none was markedly flushed.

The four graphs (fig. 1) show certain effects of the increasing temperature on the general response.

1. The systolic curve at room temperature rises slightly toward 90°. At 90°F. the curve is almost flat throughout, while at 120°F. and 130°F. it falls slightly toward the angle 90°. Whereas the systolic curve at room temperature is highest between 80° and 90° and falls as the subject is returned to the horizontal, at the elevated temperatures it is lowest between 80° and 90° and rises with the return.

2. The diastolic curve is at a lower level throughout at the higher temperatures, and the total rise is lessened.

3. The effect is to widen the pulse pressure as the temperature is steadily increased.

4. The pulse rate increased directly as the temperature.

In addition to the composite graph, a graph was prepared for subject S. G. P. (fig. 2), plotting the average of two determinations at each temperature. Although the curves are similar to those of the composite graphs, they show more extremes and more irregularity, as one would expect of an individual. These responses place him in the "Poor" group (see table 2).

Table 1a, compiled from each individual record, gives the number of significant drops in the pulse pressure at the angular intervals from 0° to

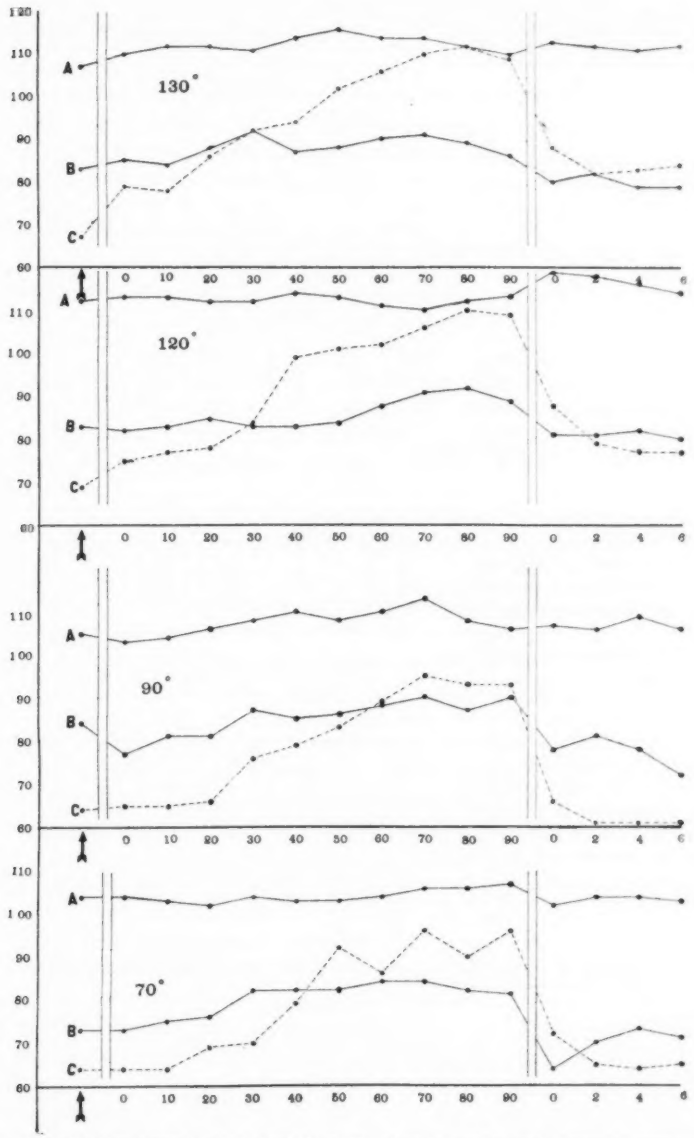


Fig. 2. Individual graph of subject S. G. P., showing pulse and pressure responses to posture at the four temperatures: 70°, 90°, 120°, 130°F. *Abscissae*, *ordinates* and *double lines* same as in figure 1. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate.

TABLE 1A

The number of significant drops in pulse pressure at the successive angular intervals

SIGNIFICANT DROPS IN PULSE PRESSURE DUE TO:	C–0	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–0	0–2	2–4	4–6
At 70°F.														
1. Fall in systolic pressure				1				1		1	1		1	
2. Rise in diastolic pressure		3	2	1		6	3	1	1			2	3	
3. Combination of 1 and 2					1									
Total number of significant drops		3	2	2	1	6	3	2	1	1	1	2	4	
At 90°F.														
1. Fall in systolic pressure	1		1		1	3	2	1	6					
2. Rise in diastolic pressure		7	4	4	3	1	3	1	2	2		2	2	
3. Combination of 1 and 2			1				1			1		2		1
Total number of significant drops	1	7	6	4	4	4	6	2	8	3		4	2	1
At 120°F.														
1. Fall in systolic pressure	2	2	3	1	4	5	2	3	5	2		2	4	
2. Rise in diastolic pressure	4	2	3	3	4	5	3	3	3	2		2		2
3. Combination of 1 and 2					1	1	2						2	
Total number of significant drops	6	4	6	4	9	11	7	6	8	4		4	6	2
At 130°F.														
1. Fall in systolic pressure	2		1	2	1	4	4		2	1		3	4	
2. Rise in diastolic pressure	1	1	3	5	5		2	4	6	1		3	2	3
3. Combination of 1 and 2		1		1	1			1		1				1
Total number of significant drops	3	2	4	8	7	4	6	5	8	3		6	6	4

TABLE 1B

Angle of occurrence of lowest pulse pressures in each experiment

TEMPERATURE	ANGLES									
	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°
°F.										
70				1		3	3	1	3	3
90		1		3	2		3	1	9	3
120		1	1	1	2	2	1	4	7	7
130	1	2	1	1		2	3	1	9	6

90° at the four temperatures. A drop of 4 mm. or more between readings was arbitrarily selected as significant. The following observations are apparent:

1. In an examination of the tables for each temperature, one is unable to locate any definite areas of predominant pulse pressure drop, but (referring again to fig. 1) one sees that the summation of the pulse pressure drops acts to bring the maximum narrowing of pulse pressure between 80° and 90°.

2. The number of significant drops in pulse pressure due to systolic pressure fall is less than the number due to diastolic pressure rise. This is also seen in the composite graph (fig. 1).

Table 1b records, at each successive angle, the number of individual experiments which had their lowest pulse pressure at that point. In the experiments with the same lowest pulse pressure at more than one angle, each lowest reading was recorded. The greatest number of lowest pulse pressures fell at the angles of 80° and 90°, as is indicated in figure 1. However, what is not shown in the graphs is that a number of individuals gave their lowest pulse pressure reading at much lower angles—30° to 40° and up.

It is well known that at the angles approaching the vertical, various mechanisms come into play to assist the return of venous blood to the right heart. Notable among these are the aspirating effect of respiration, maintenance of adequate tone in the splanchnic bed, and the compression of peripheral veins by the muscles, as a subject shifts position or tenses himself. Variations of these mechanisms are seen in persons, faint from prolonged standing, who yawn, inspire deeply, and shift their weight constantly. Although each subject was instructed to keep absolutely quiet and relaxed during the test, the question arose whether a subject in such postural experiment was not helping his venous return, unconsciously, by increased respiration, as he was being raised toward standing. To investigate this point, tests were run upon 17 subjects at room temperature and in the usual manner, but with the subject breathing through a McIlhenny and Helm gas meter. In this way the minute volume of breathing was measured during each successive 10° interval. In figure 3 are plotted the pulse and pressures in the usual form. Curve D represents the litres per minute of air being breathed at each angle by the subject. (These readings were obtained by dividing the litres of air respired in each 10° interval by the time elapsed during the interval.)

The respiration curve is practically level up to 40°, but from 40° to 90° there is a steady rise, showing the compensatory increase in ventilation which was suspected. Although the subject was returned immediately to the horizontal from 90°, the curve shows that the air respired continued to increase nearly 1 litre per minute up to 2 minutes after the return and there-

after slowly decreased. This phenomenon is perhaps a mild and temporary orthopnea, produced by the momentarily increased volume flow through the pulmonary circuit and the resultant pulmonary congestion, when the blood from the dependent parts suddenly rushed back to the right heart—a mechanism similar to that described by Field and Bock (1925) in their investigation of the orthopnea in patients with cardiac decompensation.

Since the subject was fastened to the table and used a minimum of energy in maintaining his posture even at the vertical, we considered any

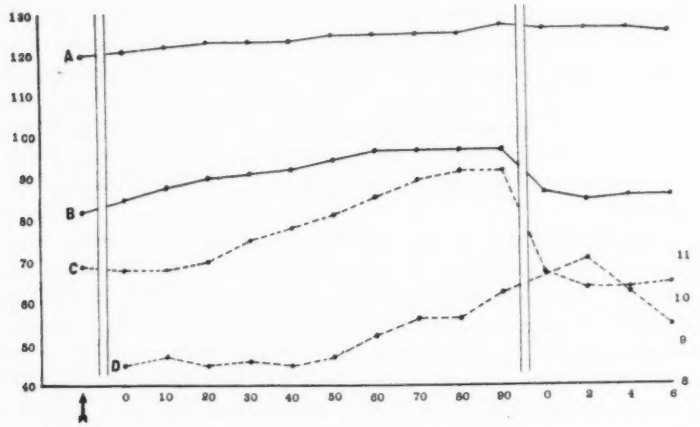


Fig. 3. Composite graph showing variation in respiratory volume accompanying postural change. *Abscissae, left ordinates* and *double lines* same as in figures 1 and 2. *Right-hand ordinates* indicate liters per minute. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate; *D*, liters per minute respired.

increase of ventilation due to increased metabolism at the upright positions to be negligible.

On the basis of the 92 tests run during this investigation and on the basis of 60 similar preliminary tests, run at a previous time by one of the authors, a few general standards have been evolved for judging what we consider good or poor responses. The results are too irregular, in our estimation, to warrant a detailed scoring system for cardiovascular efficiency, but in our judgment responses may be grouped in the following general manner:

	Good	*Poor*
1. Systolic:	a. Remaining nearly level	a. A decided fall
	b. No sharp rises or falls between readings	b. Marked variations between readings

	Good	*Poor*
2. Diastolic:	a. A gradual rise but not enough to lower the pulse pressure below 18 mm.	a. A rise lowering the pulse pressure below 18 mm.
	b. No sharp variations between readings	b. Sharp variations between readings
3. Pulse Pressure:	a. Never below 18 mm. and without any sharp variations between readings	a. Falling below 18 mm. or showing sharp fluctuations between readings
4. Pulse Rate:	a. At start not over 85 or under 45	a. At start outside these limits
	b. A moderate rise throughout the test. (In high temperatures this rise may be larger)	b. An extreme rise throughout the test
	c. No sharp variations between readings	c. Sharp variations between readings

It will be noted in this tabulation that the absence of sharp variations in any of the four readings is given a prominent position in the estimate of a favorable response. Indeed, in the absence of any extreme total variation, we regard this as the most important single item. The pressure readings, also, are regarded as of at least as much significance as the pulse rate in the cardiovascular response. Since much consideration has never been given to the return of pressure and pulse readings to constant, these data have not been included in the table.

On the basis of this table the tests were separated into a "Good" group of 19, a "Poor" group of 15, and an intermediate "Fair" group of 30. This was done to compare the estimate of good, fair, and poor with the actual "Standing" scores of the Turner scoring system, which were also taken on each subject. Table 2 shows that the average Turner score for the "Poor" group, 4.7, is definitely lower than the averages of the other two. Unfortunately, the average Turner score for the "Good" group, 7.4, is just below that for the "Fair" group, 7.9, whereas we had hoped to find it significantly larger. Both the tilting test and the standing test frequently show responses for the same subject which differ so much on different days that it is hard to conceive that the efficiency of his cardiovascular mechanism has varied to an equal degree. Both tests frequently indicate disparity of cardiovascular efficiency among a given group of subjects quite contradictory to their relative physiques. The normal limits are wide, and the tests fail to bring out any constant, clear-cut variations in response such as would justify precise grading It would seem, therefore, that these two methods of observing the cardiovascular system are but rough estimates, at best, of the true condition of the circulation, and that any attempt at a scoring more detailed than an estimation of good, fair, and poor is

artificial. It is, however, safe to say that the members of the "Good" and "Fair" groups tended to be better physical specimens and more athletic than the members of the "Poor" group.

TABLE 2

Comparison of the tilting table groups and the corresponding Turner score

GROUP	NUMBER IN GROUP	AVERAGE TURNER SCORE	DIVISION OF TURNER SCORE	NUMBER OF TILTING TABLE GROUP IN DIVISION	PER CENT OF TILTING TABLE GROUP IN DIVISION
Good	19	7.4	13–up	3	16
			10–12	4	21
			7–9	4	21
			4–6	3	16
			1–3	3	16
			0–below	2	10
Fair	30	7.9	13–up	2	7
			10–12	6	20
			7–9	13	43
			4–6	6	20
			1–3	3	10
			0–below	0	0
Poor	15	4.7	13–up	0	0
			10–12	0	0
			7–9	6	40
			4–6	6	40
			1–3	1	7
			0–below	2	13

SUMMARY

1. Tilting subjects from a horizontal to a vertical position—head up—has certain effects upon the cardiovascular response at all temperatures:

a. The systolic pressure curve remains nearly level, while the diastolic pressure steadily approaches it as the vertical position is neared. The resulting pulse pressure shows a physiological narrowing.

b. The pulse rate rises steadily to a maximum at 80° or 90°.

c. When the subjects are returned to the horizontal, the pulse pressure immediately widens in excess of the reading at the commencement of the experiment. The pulse rate rapidly drops.

2. The effects of high temperature are as follows:

a. The systolic pressure tends to fall slightly at the higher temperatures, but since the diastolic pressure curve is lowered more than is the systolic pressure curve, the pulse pressure is wider than at room temperature.

b. The pulse rate increases directly as the temperature.

c. There were three instances of fainting at 120°F. and one instance at 130°F., all occurring above the angles of 50°.

3. Tilting experiments were carried out in which the subject's ventilation was measured by means of a special spirometer. The results show a steady increase in ventilation as the subject is tilted from horizontal to vertical, a mechanism which by its aspirating effect helps to return the blood to the right heart in erect postures.

4. As regards a scoring system of cardiovascular efficiency, the writers feel that the data obtained in these experiments justify only a general estimate of any given response, such as "Good," "Fair" or "Poor."

BIBLIOGRAPHY

FIELD, H., JR. AND A. V. BOCK. 1925. Journ. Clin. Invest., ii, 67.
TURNER, A. H. 1927a. This Journal, lxxx, 601.
1927b. This Journal, lxxxi, 197.
1929. This Journal, lxxxvii, 667.
TURNER, A. H., M. I. NEWTON AND F. W. HAYNES. 1930. This Journal, xciv, 507.

THE HYDROGEN ION CONCENTRATION OF THE GASTRIC JUICE OF FETAL AND NEWBORN WHITE RATS

IRA A. MANVILLE AND ROBERT W. LLOYD

From the Department of Physiology, University of Oregon Medical School, Portland

Received for publication February 1, 1932

In the course of an investigation on the erythrocyte and hemoglobin content of fetal and newborn white rats, it was frequently noted that the stomachs of these animals were filled with a clear fluid. This fluid might represent the secreted products of the gastric glands or it might be amniotic fluid that had been swallowed. The simplest test that occurred to us to reduce these possibilities to one, was that of testing the gastric contents and the amniotic fluid for their reaction. Accordingly, the reaction was obtained first with litmus and later with standard buffered solutions of differing pH values. It was found, for example, that the gastric content of a fetus on the 18th day of gestation was distinctly acid to litmus and had a pH of 6.4 while the amniotic fluid was alkaline to litmus and had a pH of 7.4. It seemed fairly safe to conclude, therefore, that the gastric content was made up for the most part, at least, of gastric juice.

The query arose as to when the gastric juice began showing an acid reaction and how rapidly the acidity increased to that value usually found in adult animals. Data for answering the above questions were obtained in the following manner.

METHOD. Adult male and female rats of known fertility were placed on our stock diet.[1] By the use of the vaginal smear technic the period of heat was determined and the males placed with the females. The presence of sperm in a subsequent smear was considered as indicative of a positive mating. This was further confirmed if the female showed a placental leak on the fourteenth day following. The age of the fetuses was determined by this sign. As early during pregnancy as proved practicable, the female would be anesthetized and the fetuses removed one at a time by the abdominal route. The fetus' abdomen was opened and the stomach grasped by a fine curved forceps in such a manner as to include the esophagus and pylorus. As soon as the stomach was removed and while still in the grasp of the forceps, it was immersed in distilled water, rinsed and partially dried. The stomach was then held over the hollow of a micro-

[1] Cracked yellow corn, 35; cracked wheat, 35; alfalfa meal, 4; linseed oil meal, 16; casein, 5; calcium carbonate, 0.5; sodium chloride, 0.5 and dried yeast, 4.

culture slide[2] and carefully slit open. A free edge was grasped in the forceps and, by means of a fine pipette, doubly distilled water was slowly dropped over the gastric mucosa in order to wash off the gastric juice and add to the latter until there was sufficient fluid barely to fill the depression. One or two drops of an indicator were added and a cover slip sealed in place by a ring of vaseline.

A complete series of buffered solutions ranging in pH from 1 to 10 was set up.[3] A series of culture slides was placed in a row and the depressions nearly filled with those buffered solutions whose pH values would cover the range of gastric contents. The indicators used were as follows: thymol blue with a pH range of 1.2 to 3.0; methyl orange 3.0 to 4.5; methyl red 4.5 to 5.9; brom thymol blue 6.0 to 7.4 and phenol red 7.4 to 8.0. It was always necessary to sacrifice a few fetuses in order to determine the approximate range of pH and the indicator to be used. When the slides were all set up a few moments were allowed to elapse for the complete and uniform development of color. It was then only a question of matching the unknown with a known. This was a very easy procedure and one giving a high degree of accuracy as determined with small quantities of solutions of known pH. Determinations with the potentiometer (Youden) gave satisfactory agreement.

It was found impractical to work with fetuses younger than 17 days. The stomachs were too small and contained practically no fluid. The tissues were so fragile that we could not feel assured that we were not mixing tissue juice with gastric contents. For example, the stomach of a fetus removed on the 15th day gave a pH of 7.2. With the newborn animals, care had to be taken to get them before they had suckled. With those animals of greater age (from 1 to 21 days) a slightly different precaution had to be observed. Food was always present in the stomach. These animals were separated from the mother and kept in a warm place for from 2 to 4 hours before they were killed and the stomachs examined. In this way the food was considered as a test meal and the examination conducted at approximately the height of acid accumulation. In a few animals the preceding assumption was tested by injecting, previous to the examination, a solution of histamine. The solution was made up so that animals receiving 1 drop per 5 grams of body weight received 0.1 mgm. of histamine per kilogram of body weight. In the 13 trials of this nature, no values were obtained leading us to suspect that the routine procedure was giving acid values that were too low. In chart 1 the points surrounded by a circle represent the determinations obtained after the injections of histamine.

[2] Micro-culture slide 75 × 25 mm. with a cylindrical depression 3 mm. deep by 15 mm. diameter. Stock number 38230, Braun-Knecht-Heiman Co., San Francisco.

[3] We wish to express our thanks to Dr. H. D. Haskins of the Biochemistry Department for the assistance he gave us in preparing these solutions.

The Boas test for HCl was used in a few instances in post-natal rats only but because of irregular results was not continued throughout the study. Table 1 gives the results obtained. Keene and Hewer (1) used the Gunsberg reagent in testing for HCl in the stomachs of human fetuses of different ages. They tested the glycerine extract of fresh stomachs as well as the fresh material itself. Their results appear in table 2. We feel that, in our experience at least, a positive result may be considered as definitely indicative of free HCl but that a negative result cannot be considered as

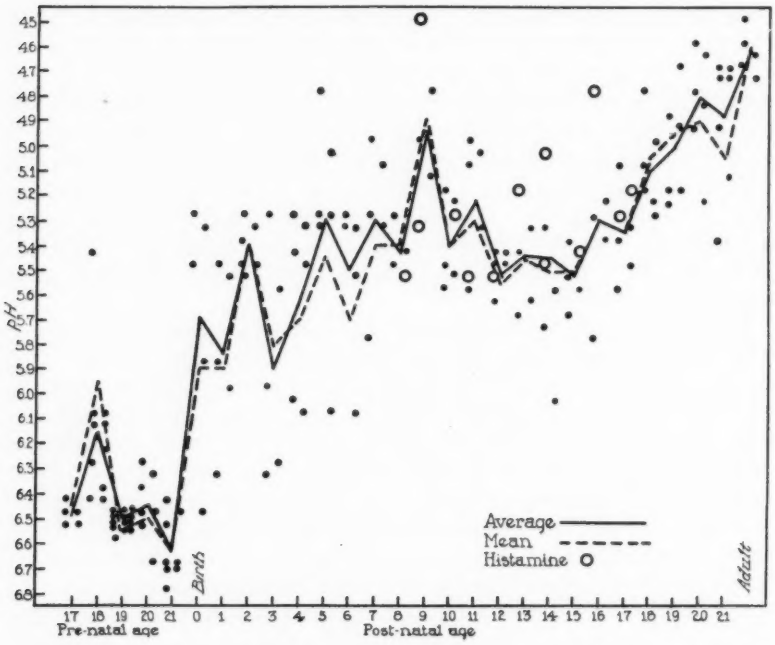


Chart 1. The pH value of the gastric juice of white rats varying in age from the 17th day of gestation to the 21st day of lactation.

conclusive evidence of the lack of HCl production. Keene and Hewer's results were not complicated by the presence of food in the stomach and still 5 out of 24 tests at full term were negative. Our negative results occurred only in the older animals where it was difficult to get them with empty stomachs.

Chart 1 summarizes the data obtained in respect to the pH of the gastric contents of fetal and newborn rats of different ages. It will be seen that during the last days of gestation there is a tendency for the gastric contents

to reach a peak in acidity and then rapidly recede so that at the end of the intra-uterine period the pH of the gastric juice was slightly greater than 6.6. Immediately after birth, however, there was a remarkable rise in acidity,—the pH changing from about 6.6 to 5.7. From this time on the changes in the pH of the gastric juice may be grouped into three phases: first, the first week of lactation where a gradual but definite increase in acidity occurs; second, the second week of lactation where a slight drop in acidity occurs, and third, the last week of lactation where there is another marked rise in acidity. At the end of lactation the pH of the gastric juice still is slightly greater than that obtained for adult animals (4.6).

TABLE 1

AGE	BOAS TEST
12 hours	2 positive
3 days	2 positive
4 days	1 positive
6 days	1 negative
18 days	2 negative

TABLE 2

AGE	GUNSBERG TEST	
	Fresh stomach	Glycerine extract
16 weeks		3 negative
17 weeks		1 negative
18 weeks	1 negative	1 negative
19 weeks	1 positive	1 negative
22 weeks	1 negative	2 negative
24 weeks	1 neg. 1 pos.	5 neg. 1 pos.
30 weeks		1 negative
32 weeks	1 positive	1 positive
Full term	8 pos. 1 neg.	11 pos. 4 neg.

Millet (2) upon determining the pH of various tissues of fetal rabbits found that "the fetal tissues become progressively less acid with increasing age of the fetus." For example, the pH of guinea pig's blood 1 hour after birth was 6.02; 3 hours later it was 6.4 and 7.5 hours later 7.16. Millet quotes Mlle. Mendeleef as observing that fetal blood of guinea pigs at half term was distinctly more acid than maternal blood. Millet himself showed the following relationship between fetal and maternal blood (table 3). Our results of 7.2 for the pH of the stomach of a fetus of 15 days and of 6.48 as the average for the 17th day indicate that prior to the 18th day for the rat, the acidity is not so marked. Furthermore, a pH of

7.4 for the amniotic fluid of an 18 day fetus shows no correspondence to the acidity of the gastric contents. It is probable, therefore, that in the rat up to the 18th day of gestation, the acidity within the gastric lumen is considerably influenced by the reaction of the tissues in general and that

TABLE 3

	DAY OF GESTATION					BIRTH
	20	23	24	26	29	
pH Heart	5.78	6.82	7.06		7.06	7.41
pH Liver	5.98	6.58	7.05	6.98	7.03	7.11
pH Kidney	5.55	6.68	6.78	6.35 6.67	6.43	7.11
pH Amniotic fluid	5.74					
pH Heart's blood			6.50	6.88		7.19*
pH Mother's blood			7.35	7.21		

* 12 hours later the pH was 7.35.

after this time the reaction is appreciably augmented by an intrinsic mechanism.

The method that we used in obtaining the pH of the gastric contents does not rule out the contributing influences that lactic acid or carbon dioxide may play. The point might well be made, therefore, that the acid pH values obtained during the period of gestation might well represent the influence of all three acids instead of HCl alone. In view of the work cited above this might be the preferable explanation. If it is true that fetal tissues during the middle of gestation are more acid than at the beginning or the termination of this period, it seems necessary to inquire into what various factors might contribute to this condition and to the decided changes which occur as gestation advances or as the result of the initiation of external respiration.

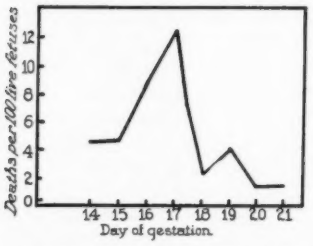


Chart 2. The death rate of fetal rats per 100 live fetuses per day of gestation.

First let us ascertain whether or not there occurs an intra-uterine crisis of serious enough nature to limit the size of the litter. A survey of our data shows that 82 pregnant females examined at varying stages of pregnancy had a total of 643 live young and 32 resorptions. Chart 2 graphically depicts the number of deaths per 100 live young by age of the fetus. The

average size of the litters was reduced from 8.6 to 7.3 during the last 8 days preceding birth. The greatest prenatal mortality was during the 17th day of gestation. Donaldson (3) shows that there is a marked decrease in growth increments of the fetus after the 17th day. It is during this period that the relatively rapid growth imposes the greatest load upon the oxygen carrying tissues. Any deficiency in oxygen transport would immediately be reflected by a stimulation of the hematopoietic tissues and the development of a condition of asphyxia the severity of which would depend upon the extent of the oxygen shortage. Products of anaerobic oxidation such as lactic acid would appear and tend to alter the reaction of the tissues. Those fetuses remaining alive through this critical period would represent either those individuals which were able to compensate sufficiently to meet the demands of the occasion or those sufficiently endowed with the essentials to maintain an existence barely within the limits compatible with life.

Toward the end of the period of gestation through the sacrifice of 15 per cent of the original litter and through the rapid development of erythrocyte and hemoglobin, the oxygen deficiency is relieved. After birth, with greatly increased oxygen tensions, the number of red cells and the amount of hemoglobin is actually reduced.

On the 9th day of lactation a gradual decrease in gastric acidity begins. The decrease reaches its lowest level on the 15th day. From this time on a gradual increase in acidity occurs. Whether or not this drop in acidity (4.94 to 5.52) represents a marked enough change to be significant is difficult to say. Mention should be made, however, of the fact that it is during this same period that the red cells and hemoglobin (especially the latter) reach the lowest level. As soon as the lactating rat begins to partake of the mother's diet there is a rapid increase in the hemoglobin and red cells. The existence of any possible relationship between the amount of acid secreted into the stomach and the oxygen carrying capacity of the blood must, for the time at least, remain an open question. It must be pointed out, however, that there is a frequent parallelism running between gastric acidity and the number of red cells and the amount of hemoglobin.

In the human fetus, Keene and Hewer (1) found that there was free HCl formed in the stomach as early as the 19th week (fresh stomachs) and the 24th week (glycerine extracts of fresh stomachs (table 3). McRobert (4) determined the pH of the gastro-intestinal tract of white rats ranging in age from 4.5 to 12 months. He found the pH of the stomach 5.5 at the cardia and 2.39 at the pylorus. Grayzel and Miller (5), working with dogs fed a normal diet, observed that the pH of the gastric contents averaged 3.68. In guinea pigs, Redman, Willimot and Wokes (6) obtained an average pH of 3.8 and in adult rats found a pH of 4.2. They carried on some investigations of the intestinal contents at various levels in young rats ranging in age from 4 to 12 weeks and found that "the small intestine

tends to be more alkaline than in adults." No determinations on the stomach were reported.

In some previous work done in this laboratory (unpublished data) on the red cell count and hemoglobin content of the blood of fetal rats as contrasted with that of the newborn and nursing rats, it was observed that there was a sharp change in level between the results obtained on the 21st day of gestation as compared wth the 1st day of lactation. It is obvious that there is an extremely marked change in living conditions in the two periods. That there should be such significant changes in some of the physiological balances existing within the body at such times is very interesting and may contribute to a better understanding of the causes which mark this time as a very crucial period in the life of the individual. Eastman (7) has shown that one of the factors contributing to make this a critical period in life is the different oxygen tensions to which the blood of the individual is exposed before and after birth. Physiology has established the fact that low oxygen tensions will cause a marked rise in the red cell count. This explains very well the marked differences in red cell counts of pre- and post-natal rats to which reference was made above. Whether or not it is a contributing factor to the detemination of the character of the gastric juice cannot be said at this time. There are those, however, who hold that there is a connection existing between the amount of carbon dioxide being eliminated from the body and the amount of HCl being produced by the gastric mucosa.

As already pointed out, the pre-natal limitation of litters occurs with the highest frequency of fetal deaths between the 14th and 18th day of gestation. It is rather common experience in the laboratory that the highest incidence of post-natal deaths occurs during the latter half of the second week and the first half of the third week of lactation. Whether or not the decrease in acidity at this time is in any way related to this occurrence remains for future work to decide.

SUMMARY

pH determinations on the gastric contents of 43 fetuses covering the last 5 days of gestation shows that during this period the acidity reaches a maximum on the 17th day and thereafter declines. Similar observations made on the gastric juice of 120 lactating rats covering the entire 21 days of the lactation period shows five noteworthy features: *a*, there is a marked increase in acidity of the gastric juice as soon as the animal is born; *b*, during the first week of lactation there is a gradual increase in acidity; *c*, the middle third of the lactating period shows a slight decrease in acidity; *d*, thc latter third of lactation shows a sharper increase in acidity than the first week; *e*, the acidity at the end of lactation is not as great as is found in the adult animal. The association of the highest incidence in pre-natal and post-natal mortality with these changes is commented upon.

BIBLIOGRAPHY

(1) KEENE, M. F. L. AND E. E. HEWER. 1929. Lancet, i, 767.
(2) MILLET, H. 1928. Journ. Biol. Chem., lxxviii, 281.
(3) DONALDSON, H. H. 1924. The rat. 2nd ed., 173.
(4) MCROBERT, G. R. 1928. Ind. Journ. Med. Res., xvi, 545.
(5) GRAYZEL, D. M. AND E. G. MILLER. 1928. Journ. Biol. Chem., lxxvii, 423.
(6) REDMAN, T., S. G. WILLIMOT AND F. WOKES. 1927. Biochem. Journ., xxi, 589.
(7) EASTMAN, N. J. 1930. Johns Hopkins Hosp. Bull., xlvii, 221.

A COMPARISON OF THE RELATION BETWEEN THE RATE OF UREA EXCRETION AND THE AMOUNT OF RENAL TISSUE IN THE DOG AND OTHER MAMMALS

EATON M. MacKAY

From The Scripps Metabolic Clinic, La Jolla, California

Received for publication January 26, 1932

Taylor, Drury and Addis (1923) have demonstrated that in rabbits there exists a linear relationship between the magnitude of the ratio: $\frac{\text{Urine urea rate}}{\text{blood urea concentration}}$ and the weight of the kidneys. A similar relationship has been found (MacKay and Raulston, 1931) in the rat and the ratio value per unit of renal weight was practically the same as that for the rabbit. The indirect figures for man are suggestive (Taylor, Drury and Addis, 1923) and it seemed possible that this ratio: renal weight figure might hold for mammals generally. A perusal of the data available for the dog however suggests that the value of the ratio: $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ per unit of kidney weight may be definitely higher than in the other species. Most of the data for the dog (Marshall and Davis, 1914; Pepper and Austin, 1915; McEllroy and Pollock, 1921) only indirectly give us information upon the point in question here. Ralli, Brown and Pariente (1931)[1] have compared the rate of urea excretion in the dog with the kidney weight but unfortunately at low urine volumes under which conditions the urea excretion: urine volume relationship is for the dog an unknown quantity. In any case the range of distribution of their kidney weights is hardly large enough to give rise to any general conclusions.

In the present experiments the conditions set down by Addis for obtaining constancy in the urea ratio for a given subject were rigidly observed. These are essentially the provoking of an adequate diuresis by water

[1] These investigators have used the term "maximum blood urea clearance" suggested by McIntosh, Möller and Van Slyke for the urea ratio as originally used by Addis multiplied by 1.67. Naturally there is an obvious advantage in uniformity of nomenclature but we have hesitated to adopt this designation for the ratio $\frac{\text{urine urea rate}}{\text{blood urea conc.}}$, as used here and in the papers of McIntosh, Möller and Van Slyke, is not the "maximum" figure which may be obtained for this measurement. It may be increased by excessive diuresis, caffeine administration, protein ingestion and other factors.

ingestion and the administration of urea three hours before observations are commenced, during fasting 12 hours after the last ingestion of food. Three hundred milligrams of urea and 10 cc. of water per kilo of body weight were first given by stomach tube and then 5 cc. of water per kilo each hour thereafter until observations were suspended. Three hourly observations were made on each animal. The dog was then killed with cyanide and the kidneys removed. After decapsulation, removal of the pelvis fat and slicing followed by blotting with filter paper to subtract as much of the adherent blood and urine as possible, the organs were weighed. The figures obtained from ten normal dogs are summarized in table 1. The constancy of the urea ratio per gram of kidney makes it obvious that as in the rabbit and rat there is a direct relationship between the kidney weight and the rate of urea excretion in the dog. The values per gram

TABLE 1

DOG NO.	BODY WEIGHT	URINE VOLUME	URINE UREA	BLOOD UREA	RATIO: URINE UREA RATE / BLOOD UREA CONCENTRATION	KIDNEY WEIGHT	RATIO PER GRAM OF KIDNEY
	kgm.	*cc. per hr.*	*mgm. per hr.*	*mgm. per 100 cc.*		*gm.*	
1	2.1	65	524	84.1	6.24	14.2	0.44
2	3.0	40	345	41.8	8.25	23.6	0.35
3	6.0	160	385	37.9	10.14	35.0	0.29
4	11.4	105	778	45.8	17.00	45.7	0.38
5	16.0	110	1,015	63.4	16.00	47.2	0.34
6	18.4	215	1,310	71.0	18.42	46.1	0.40
7	19.0	98	1,140	46.7	24.44	59.7	0.41
8	22.3	205	1,185	50.5	23.50	72.7	0.32
9	24.5	180	1,426	41.4	34.50	93.3	0.33
10	30.5	270	1,486	34.6	43.00	113.2	0.38

of kidney show however that per unit weight the dog's kidney is more active than that of the rabbit or rat. The average figure for the rabbit was 0.176 and the rat 0.198 in comparison with 0.364 per gram found here for the dog. In man this relationship obviously cannot be determined directly but it is possible to compare the urea excretion ratio with the kidney weight as predicted from body surface by methods to be published elsewhere. For comparison with the dog the expected kidney weight has been compared with the Addis urea excretion ratio in a group of normal male subjects.

The data obtained here for man and the dog are compared in figure 1 with the data of Taylor, Drury and Addis (1923) for the rabbit and MacKay and Raulston (1931) for the rat. It is evident that the activity of the kidney per gram of tissue as measured by the urea ratio is practically

the same for all four species except the dog. Here the renal activity in relation to kidney weight is significantly higher. The reason for this is not evident. We are unaware of any great differences in the structure of

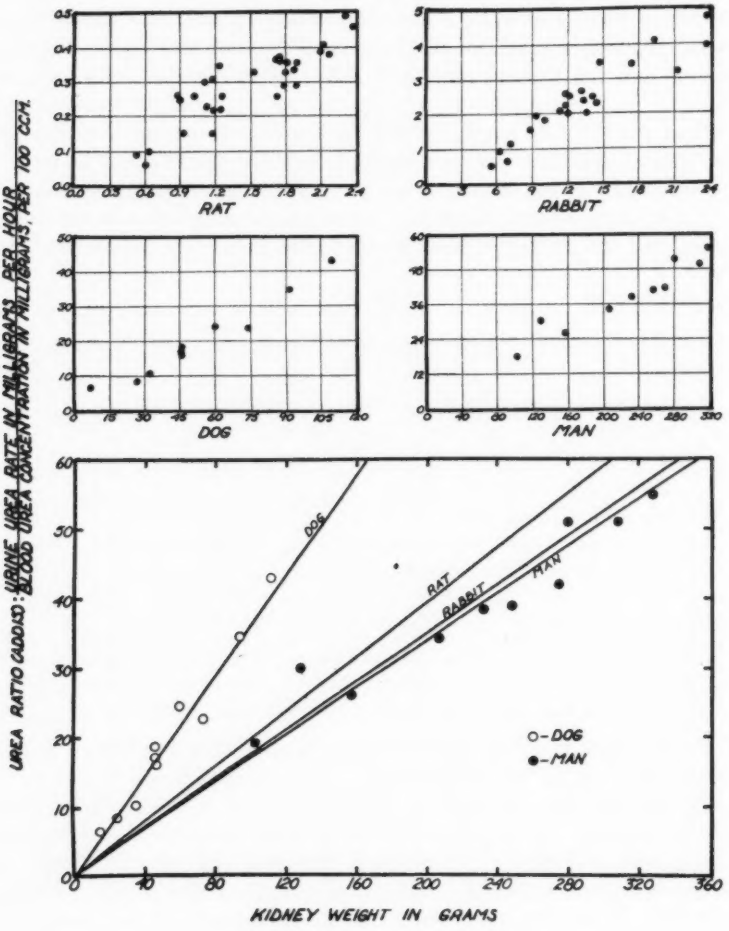


Fig. 1

the dog's kidney in comparison with this organ in the other species. We find on recalculating Vimtrup's data (1928) that the dog, man and the rat have 31,800, 31,200 and 32,200 glomeruli per gram of kidney respectively. Kunkel (1930) finds a diameter for the renal glomeruli in the dog

of 186 micra in comparison with 151 micra for the rat and 142 micra for the rabbit but records only 13,300 glomeruli per gram of dog kidney in contrast with 33,000 and 31,000 respectively for the rabbit and rat.

It occurred to us that the dog's renal tubules may absorb less of the glomerular filtered urea than appears to be taken up by those of the frog (MacKay and Oliver, 1930) or rabbit (MacKay and MacKay, 1930). If we consider the possibility that Rehberg's (1926) contention that creatinine reaches the urine solely by glomerular filtration and that none is reabsorbed in the tubules is correct, we may examine this question through a simultaneous comparison of urea and creatinine ratios. This was done in a number of dogs with results essentially the same as those since reported by Jolliffe and Smith (1931a). The creatinine ratio is definitely higher than the urea ratio in the dog as in the rabbit (MacKay and Cockrill, 1930) and man (MacKay, 1930). The only other possibility which suggests itself is that man, rabbit and rat are either omnivorous or herbivorous while at least in our material the dog is entirely carnivorous and it has been shown (Addis and Drury, 1923) that protein increases the $\frac{\text{urea ratio}}{\text{kidney weight}}$ relationship transiently when administered during the period of observation. The effect of the maintenance diet preceding the observation is unknown. Jolliffe and Smith (1931b) have found that for a given dog the urea ratio fluctuates with the amount of protein in the diet. This however aids us little for it is probable that the renal weight of the dog depends upon the protein intake just as in the rat (MacKay and MacKay, 1931). Observations upon this point and the effect of the maintenance diet preceding the measurement of the urea ratio upon the $\frac{\text{urea ratio}}{\text{kidney weight}}$ relationship are now in progress.

Since in all of the four species examined here there is a direct relation between the kidney weight and urea excretion ratio it follows that the same thing is true between the ratio and body surface for it has been shown by Stewart (1921) for the dog, Taylor, Drury and Addis (1923) for the rabbit and, MacKay and MacKay (1927) for the rat and elsewhere (MacKay, 1932) for man that the kidney weight bears a direct relation to body surface.

SUMMARY

The urea excretion ratio: $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ bears a direct relation to renal weight in the dog, rat, rabbit and man. In the dog the ratio value per gram of kidney weight is a great deal higher than for any of the other three species examined in all of which the relationship is essentially the same. The possible reasons for this are discussed.

BIBLIOGRAPHY

ADDIS, T. AND D. R. DRURY. 1923. Journ. Biol. Chem., lv, 629.
JOLLIFFE, N. AND H. W. SMITH. 1931. This Journal, xcviii, 572; xcix, 101.
KUNKEL, P. A., JR. 1930. Bull. Johns Hopkins Hosp., xlvii, 285.
MACKAY, L. L. AND E. M. MACKAY. 1927. This Journal, lxxxiii, 191.
MACKAY, E. M. AND J. OLIVER. 1930. Journ. Exper. Med., li, 161.
MACKAY, E. M. AND L. L. MACKAY. 1930. Journ. Exp. Med., li, 609.
MACKAY, E. M. AND J. R. COCKRILL. 1930. This Journal, xciv, 220.
MACKAY, E. M. 1930. Proc. Soc. Exp. Biol. and Med., xxvii, 524.
1932. Arch. Int. Med., to be published.
MACKAY, E. M. AND B. O. RAULSTON. 1931. Journ. Exper. Med., liii, 109.
MACKAY, E. M. AND L. L. MACKAY. 1931. Journ. Nutri., iii, 375.
MARSHALL, E. K., JR. AND D. M. DAVIS. 1914. Journ. Biol. Chem., xviii, 53.
MCELLROY, W. S. AND H. O. POLLOCK. 1921. Journ. Biol. Chem., xlvi, 475.
MCINTOSH, J. F., E. MÖLLER AND D. D. VAN SLYKE. 1928. Journ. Clin. Invest., vi, 467.
PEPPER, O. H. P. AND J. H. AUSTIN. 1915. Journ. Biol. Chem., xxii, 81.
RALLI, E. R., M. BROWN AND A. PARIENTE. 1931. This Journal, xcvii, 432.
REHBERG, P. B. 1926. Biochem. Journ., xx, 447.
STEWART, J. N. 1921. This Journal, lviii, 45.
TAYLOR, F. B., D. R. DRURY AND T. ADDIS. 1923. This Journal, lxv, 55.
VIMTRUP, B. 1928. Amer. Journ. Anat., xli, 123.

CAPILLARY COUNTS IN RESTING AND ACTIVE MUSCLES[1]

E. G. MARTIN, EOLA C. WOOLLEY AND MIRIAM MILLER

From the Laboratory of Physiology, Stanford University

Received for publication January 19, 1932

In the course of a study in this laboratory of the activity metabolism of single mammalian muscles in situ (Martin, Field, Hall and Field, 1931) it seemed desirable to have precise information of the details of the capillary circulation in the muscles under various conditions, and especially in rest and during exercise. The important pioneer work of Krogh on the capillary circulation of muscles (1922) was done on muscles other than the gracilis (in the dog the muscle examined by Krogh was the semimembranosus) whereas the work in this laboratory has been done on the gracilis, and it is quite conceivable that findings from one muscle might not hold closely for another, even in the same animal. We accordingly undertook the series of observations reported in this paper. All the work was done on gracilis muscles of dogs, except in one experiment in which the semimembranosus muscles were used to enable us to compare the results obtained by our method with those obtained by Krogh, who used a somewhat different injection technique.

Methods. We made the capillaries demonstrable by flooding the intact muscle in situ with undiluted india ink under the arterial pressure prevailing at the instant of injection, the pressure being recorded throughout each experiment from a cannula in a carotid artery. The gracilis muscle of the dog lends itself admirably to this procedure, both on account of its own superficial position and because of the ready accessibility of its main nutrient vessels. A cannula directed centrally was introduced into the femoral artery just distal to the point of origin of the gracilis artery. A syringe of about 15 cc. capacity, filled with india ink, was connected to the cannula, air bubbles being carefully excluded in making the connection. At the desired moment a clip that had been previously placed on the syringe tube was removed and a considerable volume of ink injected into the femoral artery. The rate of injection was so adjusted as to insure that the blood entering the muscle was wholly or almost wholly replaced by ink. Both the muscle and the venous outflow blackened promptly, and as soon as the latter blackening was clearly evident, a matter of ten seconds or

[1] A preliminary report on part of this work was published in the Proceedings of the Society for Experimental Biology and Medicine, 1930, xxviii, 33.

thereabouts, the gracilis artery and vein were clamped off, as were also both ends of the muscle by means of large hemostats previously placed in position. The muscle, which had been freed from the adjoining tissues except at origin and insertion prior to the injection, was then quickly cut away, outside the hemostats, and with the aid of the latter plunged into a large beaker of Bouin's fluid for fixation. The muscle shortens and thickens somewhat when cut away from its attachments, and we endeavored to compensate for this to some extent by holding the hemostats apart and the muscle thus under some tension during the first few minutes of fixation. It is not likely that we duplicated the normal tension but we were successful in avoiding distortion of the muscle, our fixed preparations showing, practically without exception, straight fibers throughout.

After preliminary fixation of about an hour the muscle was cut into strips of suitable size for sectioning and the fixation continued for about twenty-four hours. The standard procedure for making paraffin sections was then followed. Transverse sections were cut in thicknesses of nine to fifteen microns, enough being made from the different strips to ensure that all parts of the muscle were sampled. In all the preparations from animals in which amytal was used as the anesthetic (the technical work on these was done by E. W.) the sections were stained on the slides with Delafield's hematoxylin and eosin. Twenty-two muscles were treated thus. In the preparations from etherized dogs, decerebrate dogs, and dogs into which amyl nitrite was injected (the technical work on these was done by M. M.), the sections were mounted without staining. We found, as a matter of fact, that by discontinuing the washing with alcohol before the last traces of yellow coloration from the Bouin's fluid had disappeared we obtained sections in which all the features significant for our purpose, i.e., the boundaries of the fasciculi, the outlines of individual fibers, and the injected capillaries, stood out more sharply than in the stained preparations. Eleven muscles were treated thus.

The method of demonstrating capillaries by india ink injection has been criticized by Hartman, Evans and Walker (1929) on the ground that ink particles have been observed by them to catch at the origins of open capillaries, preventing ink from entering them and thus excluding them from subsequent counts. We are, of course, unable to say to what extent this may have happened in our injections. We endeavored to minimize it by flooding the muscles with ink, as described above, and also to reduce its effect by taking the averages of counts from a great many fields from each preparation. On the basis of our rather extensive experience we doubt that it occurs on a large enough scale to impair the validity of results obtained by the ink injection method.

Fasciculi and muscle fibers. In the gracilis muscle of the dog the fibers are grouped in well marked fasciculi, usually triangular in cross section in

fixed preparations. The average number of fibers in 140 fasciculi selected at random was 66. No fasciculi were observed with fewer than 30 fibers and none with more than 125. The mode of the distribution curve was at 60 fibers, and sixty per cent of the fasciculi contained between 45 and 70 fibers. A number 10 ocular and 4 mm. objective were used, the former equipped with a micrometer. Fiber counts were not made in the early experiments (those by E. W.). In the later experiments (by M. M.) in which fibers were counted the micrometer field used was a rectangle 69 microns long and 34.5 microns wide. In two hundred such fields from one muscle the average number of fibers per field was 4.12. In only two fields were there fewer than three fibers in the field, and in only fourteen were there more than five fibers in the field; ninety-two of the two hundred fields contained four fibers. This uniformity of cross section of fibers is fully as great as one would expect to find in view of the fact that some of the fields are bound to contain fibers sectioned near their ends, where they have begun to taper. The average cross sectional area of these fibers was 0.58×10^{-3} sq. mm., and the average diameter 28 microns. The dog from which this muscle was taken was full grown, weighing about 12 kgm. Another muscle, from a dog weighing 21 kgm., was composed of considerably larger fibers, the average cross section of these being 0.77×1^{-3} sq. mm., and their average diameter 32 microns. In a third dog, a puppy weighing 6 kgm., the average fiber cross section was only 0.44×1^{-3} sq. mm., and the average diameter only 24 microns. There was also considerably greater variation in size of fibers in this muscle from a puppy than in the muscles obtained from full grown animals. Fiber counts were made on six gracilis muscles in addition to the three described above; the values from all six being intermediate between the second and third of those described. In terms of number of fibers per square millimeter of cross section our figures for gracilis muscles from full grown dogs range from 1291 to 2012, averaging 1640. This latter average implies an average cross section of individual fibers of 0.61×10^{-3} sq. mm. and an average fiber diameter of 29 microns.

It should perhaps be pointed out that so far as these measurements may be in error the error is almost certainly in the direction of greater apparent fiber cross section, and hence of fewer fibers per sq. mm. of muscle cross section than exist in the intact organism. The fixative used by us (Bouin's fluid) is stated by experienced histologists not to induce shrinkage of muscle tissue, and we saw no evidence of such shrinkage in our preparations. Since our muscles were fixed under less tension than prevails in situ (see p. 408) the only possible departure, therefore, from the dimensions of the intact muscle is in the direction of fiber thickening.

The capillary supply of the gracilis muscle. Following the example of Krogh (1919) we injected the gracilis muscle in one dog after dilating the

vessels with amyl nitrate. This dog, weighing 21 kgm., was anesthetized with "nembutal" (sodium ethyl (1 methyl-butyl) barbiturate). It showed an arterial pressure, prior to administration of amyl nitrite, of 134 mm. Hg. A small quantity of the latter drug was introduced intravenously, whereupon the arterial pressure fell abruptly to 18 mm. Hg. Shortly after this level was reached ink was injected according to the method described near the beginning of this paper. The muscle became very black, and when sectioned showed a very uniform distribution of capillaries. The average number per sq. mm. of cross section was 2580, thus agreeing closely with the findings of Krogh on the semimembranosus of the dog. The average number of muscle fibers per sq. mm. of cross section in this muscle was 1291, consequently there were two capillaries, on the average, for each muscle fiber. As a check on this ratio, which seems to us important, we compared the ratios of capillaries to muscle fibers in two microphotographs of injected horse muscle published by Krogh. In one of these (1919) the ratio is approximately 2.6, and in the other (1922) approximately 2.0. Paff (1930) using a standard injection mass, instead of india ink, obtained in a series of eight muscles from rat, guinea pig and cat an average ratio of fibers to capillaries of 2.6 (calculated by us from his table). In four of his eight (including all three of his cat muscles) the ratio ranged between 1.81 and 1.92. We were able to obtain a microphotograph of a particularly heavily injected area from the gracilis on which our counts were made, and this area showed a ratio of approximately 2.5. We thus have a fairly definite picture of the potential blood supply of individual muscle fibers in the gracilis muscle, the usual distribution being of the order of two capillaries to the fiber, and two and a half to the fiber the approximate maximum in the most heavily vascularized areas.

Capillary counts in resting muscles. One gracilis muscle was injected with india ink in each of eight full grown dogs, as early as possible after complete anesthetiziation, and with the animals quiet. In five of the experiments amytal was used as the anesthetic, in two morphia and ether, and in the remaining one, decerebration under transient etherization.

A striking observation, and one that seems to us probably significant, is that in all these muscles fasciculi that showed no injected capillaries, or at most a very few, were interspersed among well-injected fasciculi. The fasciculi in which the capillaries were devoid of ink were practically always penetrated by larger vessels in which ink was present, hence the failure of these capillaries to take ink cannot be accounted for on the assumption that they sprang from a different arterial source. Nor would the well-known richness of anastomosis among the intramuscular vessels justify such an assumption. In one muscle, which we examined with some care, approximately sixty per cent of the fasciculi showed characteristic injection of capillaries and forty per cent absence of capillary injection. The obvi-

ous suggestion is that the rotation of function among the capillaries postulated by Krogh (1919) operates in terms of whole fasciculi, which alternate in receiving the blood-flow characteristic of resting muscles and in being virtually deprived of blood for a time.

Capillary counts were made in injected areas, and in six of the eight muscles under discussion these were very consistent, ranging between averages of 917 and 1154 per sq. mm. for individual muscles, with an average for the group of 1056 per sq. mm. The other two muscles (the first two of the series) showed markedly fewer capillaries, each averaging 770 per sq. mm. We consider the consistent showing of the group of six muscles to be probably more nearly characteristic of gracilis muscles in general, than the smaller figures, and consequently look upon the value 1050 as the most probable approximate average number of open capillaries per square millimeter in such fasciculi of resting gracilis muscles as are receiving blood. The average number of muscle fibers per sq. mm. is stated above (p. 409) to be 1640, hence the ratio of open capillaries to fibers is 0.64. The puppy on which observations were made (p. 409) showed an average of 1690 capillaries per sq. mm. cross section, a value more than 50 per cent greater than the average given above. Its muscle fibers were smaller, however, than the corresponding fibers in full grown dogs, consequently the ratio of open capillaries to fibers was less disproportionate, being 0.74, as compared with 0.64 for the full grown animals.

Capillary counts in muscles injected during exercise. In each of eight dogs one gracilis muscle was prepared for injection in the manner described, and then stimulated rhythmically through the obturator nerve by brief tetanic shocks repeated about once a second. Stimulation was continued for three to five minutes to give time for full adjustment of the intramuscular circulation to the exercise state. Ink was then injected *while the exercise was still in progress,* and the subsequent procedures carried out as described. The capillary counts were reasonably consistent, the averages from the different muscles ranging from 1708 to 2437 per sq. mm., with an average for the series of 2010 per sq. mm. The ratio of this average to the average number of fibers per sq. mm., 1640, is 1.23, which is approximately twice the ratio found in resting muscles. It is, however, well below the ratio found in muscles treated with amyl nitrite (p. 410), so that while exercise by direct stimulation about doubles the number of open capillaries in individual areas it does not appear to cause all that are present to open, or at least to open widely enough to take ink.

Even in these exercised muscles there were occasional fasciculi with uninjected capillaries interspersed among the fasciculi that had taken ink in characteristic fashion. The proportion was much smaller, however, than in resting muscles. In an effort to discover whether the proportion of fasciculi with open capillaries increases progressively as the exercise pro-

ceeds, as well as to discover whether the increase in number of open capillaries in other fasciculi is also progressive during exercise, two more muscles were injected, one after exercise had been under way for about one minute, the other as soon as convenient after stimulation was begun, probably about 15 to 20 seconds after the first contraction. The ratio of capillaries to fibers was 1.12 in the first of these and 0.91 in the second, thus indicating quite clearly that the capillaries open progressively during the early stages of exercise. A careful estimate of the proportion of fasciculi with uninjected capillaries was made in the second of these two muscles. Approximately thirty per cent of the fasciculi were in this state, indicating that the proportion of fasciculi receiving blood during exercise also increases progressively.

Capillary counts in muscles injected late in experiments and under different arterial pressures. It was a matter of interest to discover whether a definite relationship exists between general arterial pressure and the number of open capillaries per sq. mm. of muscle cross section. The experiments thus far described failed to throw light on this question because the arterial pressures did not vary materially. They approximated 100 mm. Hg in all the significant experiments, in no case falling below 90 mm. Hg, nor exceeding 120 mm. Hg. We have a significant series, however, consisting of six injected muscles from animals that had been subjected to drastic experimental treatment and had been under the influence of amytal for at least an hour and a half. Two of these animals had been curarized; one had had both phrenic nerves cut; the remaining three had had vigorous artificially induced exercise of all the main muscles for fifteen minutes followed by a recovery period of at least an hour. The arterial pressures and capillary counts are given in the subjoined table.

ARTERIAL PRESSURES	CAPILLARY COUNTS PER SQUARE MILLIMETER
35	1,496
38	1,877
60	1,851
80	1,903
137	1,703
142	1,782

With a single exception the counts fall within a narrow range although the differences in arterial pressure are extreme. We see no indication that arterial pressure as such is a significant determinant of the capillary count. Either the prolonged anesthetization or the treatment to which the animals were subjected, or both in combination operated, however, to cause capillaries to open to an extent not materially below that induced by exercise.

Capillary counts in muscles injected post-mortem. We considered it of

interest to determine whether the capillary counts in muscles injected post mortem without previous dilatation with amyl nitrite would approximate more nearly the counts in muscles injected during exercise or in muscles injected after amyl nitrite. Accordingly both gracilis muscles were prepared for injection in each of three dogs. Instead of injecting ink into the blood stream immediately, however, the femoral artery on one side was tied above the origin of the gracilis artery, and the vessels of the muscle flushed out with Ringer's fluid, which was introduced under moderate pressure through the cannula which had previously been placed in position for making the ink injection. Washing with Ringer's fluid was continued until no red color could be detected in the fluid escaping through the vein. The muscle was then injected with undiluted india ink under moderate pressure. The interval between the stoppage of the circulation and the injection of ink was about a half hour. The same procedure was followed on the opposite side of the body except that as soon as the flushing with Ringer's fluid was completed the muscle with its connecting vessels, and with the cannula in position, was dissected out and placed on ice for 24 hours, and was then injected with ink.

The average capillary counts for the three muscles injected one-half hour after stoppage of the circulation were 1981, 2266, and 2310, the mean being 2185, and for the three muscles injected after twenty-four hours on ice, 1730, 1756, and 1877, the mean being 1790. It was noteworthy that no uninjected fasciculi were seen in any of these muscles, the distribution of capillaries being very uniform over all parts of the sections.

It will be noted that the capillary counts from these muscles approximate more nearly the counts from muscles injected during exercise than those from muscles after amyl nitrite. The possible significance of this finding will be considered in the discussion.

Observations on semimembranosus muscles. In a 16 kgm. dog, anesthetized with "nembutal," and with all muscles at rest the semimembranosus muscle on one side was injected with ink, the arterial pressure at the moment of injection being 96 mm. Hg. Amyl nitrite was then introduced intravenously. The arterial pressure fell to 40 mm. Hg, and at this pressure the semimembranosus on the opposite side was injected with ink. The injection technique was similar to that described for the gracilis, the main artery to the muscle being flooded with ink from a side branch, after the vessels to other muscles than the one to be injected had been tied.

To our surprise the fibers of this muscle proved to average much smaller than in any of the gracilis muscles examined. The fibers of the first muscle averaged approximately 4400 per sq. mm. of cross section; corresponding to an average fiber cross section of about 0.23×10^{-3} sq. mm., and an average fiber diameter of 17 microns. The fibers of the second muscle averaged approximately 3800 per sq. mm. of cross section, corresponding to an aver-

age fiber cross section of about 0.26×10^{-3} sq. mm., and an average fiber diameter of 18 microns.

The capillary counts were reasonably consistent with expectations based on our observations on gracilis muscles. Thus, the average count in the resting semimembranosus was 3240 per sq. mm., the ratio to fiber count being 0.74, as compared with an average of 0.64 in gracilis muscles under corresponding conditions. This muscle showed an alternation of injected with uninjected areas, similar in general to the alternation seen in resting gracilis muscles, although there was much less uniformity in the distribution of injected areas in relation to uninjected, a uniformity which was usually quite marked over the whole of the resting gracilis. The average count of capillaries in the muscle injected after amyl nitrite was 5900 per sq. mm. The ratio to fiber count in this case was 1.55, considerably smaller than the corresponding ratio in the gracilis, but still indicating a more extensive opening of capillaries than we have observed under any treatment other than amyl nitrite. The fact that our average capillary count is so much higher than Krogh's on a corresponding muscle we attribute to an individual variation, since if the fibers in this muscle had been of average size, and the capillary-fiber ratio unchanged, the capillary count would have agreed satisfactorily with that reported by Krogh.

Discussion. The observations on resting muscle reported in this paper appear to us to support the conception of the capillary circulation advanced by Krogh except for the detail that it indicates the blood supply to such parts of the resting muscle as are receiving blood to be much more copious than was suggested in his postulate. In this respect our findings corroborate those of Hartman, Evans and Walker on cats' muscles (1929). The fact that every resting muscle examined by us showed areas devoid of open capillaries interspersed among areas with open capillaries can best be explained in terms of Krogh's idea of a rotation of function among the capillaries. The observation that the fasciculi appear to be the circulatory units in that they tend either to show the blood supply characteristic of resting muscles or to be practically devoid of blood in their capillaries, is an interesting anatomical detail which does not appear to us at the moment to have any special significance.

We desire to call particular attention to the fact that under four quite diverse conditions, i.e., muscular exercise (after adjustment of the circulation has had time to take place), in the last stages of drastic and prolonged experimentation upon the entire animal, soon after washing the blood out of the vessels of the muscle and probably before actual death of the muscle cells, and finally, after excision and exposure to cold for twenty-four hours, the capillary counts fall consistently within a fairly narrow range, of which the mean value is virtually twice the mean capillary count in blood-receiving areas of resting muscle. This consistency seems to us to be too marked

to be accidental. It is most simply explained as merely the result of relaxation of the walls of all the capillaries to what might be termed a resting position, or at least one in which constrictor influences are absent. In other words, we offer the suggestion that the dilatation of the capillaries observed under the four conditions described is due to the abatement of influences which tend to cause capillary constriction, rather than to the onset of positive capillo-dilator influences. A definite argument in favor of this idea is that it is obviously the most likely explanation in the case of muscles injected post mortem.

If this suggestion is to merit serious consideration it must account for the fact that after treatment with nitrites a considerably larger number of capillaries can be injected than under any of the above conditions. In explanation we would revive an idea voiced some years ago by Cohnstein and Zuntz (1888), that every capillary field contains numbers of capillaries whose cross-section, even in the relaxed state, is so small that they will not permit corpuscles to pass, and hence, as Krogh showed (1919), will not take ink. Our data do not support the idea of these authors that they open out when the pressure becomes high. Under the action of a powerful capillary dilator like amyl nitrite we presume them, however, to dilate beyond their "resting" state, thus acquiring a sufficient caliber so that they can be injected. This suggestion carries the implication that these particularly small capillaries will never be traversed by corpuscles under "physiological" conditions. They may or may not be closed completely under circumstances in which the majority of capillaries are in a semiconstricted state, but their function can not extend beyond that of transporting plasma, even when in a state of "normal" dilatation.

In his extensive discussion of capillary function, Hooker (1921) cites observations which suggest strongly that individual capillaries take on a greater caliber under the influence of powerful capillary dilators than they can be caused to take on by any other means. These observations harmonize with our idea that the exceptionally high capillary counts after nitrites are due to the resultant widening of capillaries that are normally too narrow, even when free from constrictor influence of any sort, to take either red corpuscles or ink.

SUMMARY

1. In the resting gracilis muscles of dogs the fasciculi appear to be the circulatory units in the sense that in the "rotation of function" among the capillaries, typical of skeletal muscles, the capillaries of individual fasciculi tend either to be closed en masse or to be open to an extent characteristic of muscles in a state of rest. When so open the ratio of number of open capillaries to number of muscle fibers averages about 0.64. Capillaries are designated as open when they will take india ink upon mass injection of ink into the vessels of the muscle.

2. Under the following conditions, i.e., during exercise, toward the end of long and drastic experiments on the entire animal, and both early and late after stoppage of the circulation, the number of open capillaries averages about double the number in injected fasciculi of resting muscle. A few uninjected fasciculi are seen in muscles injected after four to five minutes of exercise, none in muscles under the other conditions described.

3. The marked agreement among the capillary counts under the diverse conditions cited in the above paragraph is interpreted to mean a general relaxation of all the capillaries to a "resting" state. The abatement of influences tending to cause capillary constriction is considered to account for this relaxation rather than the onset of positive capillo-dilator influences.

4. After administration of amyl nitrite the ratio of number of open capillaries to number of muscle fibers ranges between 2 and 2.5, as compared with about 1.2 for the corresponding ratio under the conditions cited in paragraph 2. To explain this marked increase in number of open capillaries an old assumption is revived, to the effect that a considerable number of capillaries are present in addition to those that can be injected under the conditions noted in paragraph 2, these additional capillaries being of such small caliber that they cannot be injected even when normally relaxed, but under the influence of powerful capillo-dilators will relax still further, to a caliber at which they will take ink.

5. In terms of number of units per sq. mm. of cross section the following are the average data for gracilis muscles of the dog, as reported in this paper. Muscle fibers, 1640; open capillaries after amyl nitrite, 2580; in injected areas of resting muscle, 1050; during muscular exercise, 2010; in the late stages of long experiments, 1820; a half hour after replacing the blood with Ringer's fluid, 2185; twenty-four hours after excision of the muscle, 1790.

BIBLIOGRAPHY

COHNSTEIN AND ZUNTZ. 1888. Pflüger's Arch., xlii, 303.
HARTMAN, EVANS AND WALKER. 1929. This Journal, xc, 668.
KROGH, A. 1919. Journ. Physiol., lii, 409.
1922. The anatomy and physiology of capillaries. New Haven.
MARTIN, E. G., J. FIELD, 2nd, V. E. HALL AND S. M. FIELD. 1931. This Journal, xcvii, 545.
PAFF, G. H. 1930. Anat. Rec., xlvi, 401.

REFLEX STIMULATION OF RESPIRATION FROM INCREASE IN VENOUS PRESSURE

T. R. HARRISON, W. G. HARRISON, JR. AND J. P. MARSH

From the Department of Medicine of the Vanderbilt Medical School

Received for publication February 5, 1932

During some studies on cardiac dyspnea it was found that persons with cardiac disease reacted to muscular exercise with a greater increase in ventilation than did normal subjects. As neither group of individuals exhibited alterations in the composition of the blood (Cullen, Harrison, Calhoun, Wilkins and Tims) it seemed that the cause of this phenomenon had to be sought elsewhere. Schott has shown that muscular effort causes a greater and more lasting rise in the venous pressure of such patients than in that of normal subjects. Bainbridge demonstrated that rise in venous pressure causes reflex acceleration of the heart. It seemed possible that there might be a similar reflex effect on respiration and the present study was made in order to test this hypothesis.

Our observations were made upon dogs anesthetized with barbital. Respirations were recorded with a Benedict spirometer. In the first group of observations fluid—either Ringer's solution, normal saline solution, or defibrinated blood—was injected rapidly into the external jugular vein or into the inferior vena cava. Examples of such experiments are shown in figure 1. Well marked increases in respiratory rate and in minute ventilation resulted when the vagus nerves were intact. After bilateral vagotomy, no change or only slight increase in respiration was observed when fluid was injected.

In order to determine whether the acceleration of breathing was related or not to a temporary plethora *per se*, other experiments were done in which fluid was injected into the external jugular vein low in the neck, or the inferior vena cava high in the abdomen, the cannula pointing away from the heart. By this means it was possible to produce plethora and increase in pressure in large veins without causing increase in pressure in the right auricle or in the veins adjacent to it. Under such conditions injection of similar amounts of fluid was entirely without effect on respiration.

A second group of experiments was then performed. A rubber balloon, improvised from a rubber catheter and a condom, was passed into the right auricle through either of the veins already mentioned. Air could be injected into or withdrawn from the balloon at will. The results of such an

experiment are shown in figure 2. When as little as twenty cubic centimeters of air were introduced into the balloon there was a progressive increase in respiratory rate. Deflation of the balloon caused the reverse change. After bilateral vagotomy similar but much less striking changes were noted in some dogs while in others no effect was observed. When the vagus nerves were intact these changes occurred in every instance.

These experiments seem to indicate that elevation of the venous or intra-auricular pressure causes reflex stimulation of breathing, the reflex arising

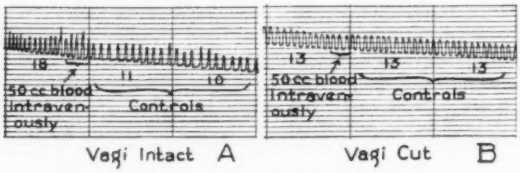


Fig. 1. The curve runs from right to left

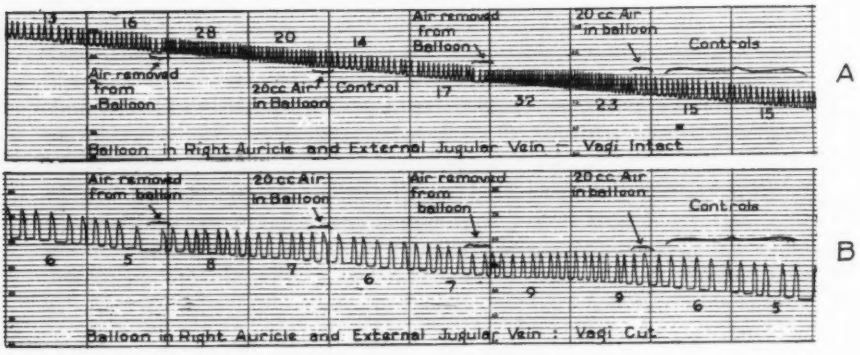


Fig. 2. The curve runs from right to left

either in the heart or in the great veins very close to the heart, and the vagus nerves being the main afferent path. The observations are in accord with those of Heymans and Heymans who observed, in cross circulation experiments, that changes in the pressure of the blood in the heart caused reflex changes in breathing. Somewhat similar findings were reported by Sutton and Lueth who found that distention of the left ventricle produced dyspnea in the dog. It seems likely that both of the experiments reported by these authors and also our observations are of some importance in explaining the respiratory response to muscular exertion, and

especially so in regard to the abnormally great increase in ventilation which occurs during exercise in persons with cardiac disease.

SUMMARY

Increase in the venous pressure produced either by rapid intravenous injection of fluid, or by distention of a balloon in the right auricle is accompanied by increase in the respiratory rate and in the ventilation of dogs with intact vagi. The same procedures were usually without effect after double vagotomy.

BIBLIOGRAPHY

BAINBRIDGE, F. A. 1915. Journ. Physiol., 1915, i, 65.
CULLEN, G. E., T. R. HARRISON, J. A. CALHOUN, W. E. WILKINS AND M. M. TIMS. 1931. Journ. Clin. Invest., x, 807.
HEYMANS, C. AND J. F. HEYMANS. 1926. Compt. Rend. Soc. de Biol., xcv, 1118.
SCHOTT, E. 1912. Deutsch. Arch. f. Klin. Med., cviii, 537.
SUTTON, D. C. AND H. C. LUETH. 1930. Arch. Int. Med., xlv, 827.

HUMAN MILK STUDIES

XI. Vitamin G (B_2) Content of Mixed Milk[1,2]

EVA DONELSON and ICIE G. MACY

From the Nutrition Research Laboratories of the Merrill-Palmer School and the Children's Hospital of Michigan, now the Research Laboratory of the Children's Fund of Michigan, Detroit

Received for publication January 21, 1932

Clinical observations (1–4) and animal experimentations (5–8) indicate that both mother's and cow's milk have a relatively low potency in the vitamin B complex[3] and at times may be insufficient to satisfy the vitamin demands of the growing young when milk is the sole food supply. The vitamin B complex content of breast milk is dependent on the richness of the maternal dietary in this vitamin moiety and on the extent to which it is used up in the ordinary maternal bodily functions and in the additional processes of milk secretion.

Since human milk is the natural food for the infant during the first months of life, its evaluation in the known dietary components is of importance. With a knowledge of the amounts of the various food principles in mother's milk, it is possible to provide the young with a diet adequate for growth and well-being, since the necessary supplements can then be provided either by direct administration to the child's diet or by reinforcing the milk through the maternal dietary (9–11). Adequate nourishment during early life is one of the fundamentals in the achievement of good bodily structure and a stable functional capacity.

[1] This study was kindly aided by a grant from the Committee on Scientific Research of the American Medical Association. A preliminary report was presented before the Division of Biological Chemistry at the 82nd meeting of the American Chemical Society in Buffalo, August 31 to September 4, 1931.

[2] We wish to express our thanks and appreciation to Maurice H. Givens, Ph.D., of the Northwestern Yeast Company, Chicago, for the yeast used in this study, and to Arthur D. Holmes, Ph.D., of the E. L. Patch Company, Boston, for the cod liver oil. It is a pleasure to acknowledge the continuous interest and coöperation of the Mother's Milk Bureau of Detroit.

[3] In this report the old vitamin B that is now known to be composed of several fractions will be referred to as the vitamin B complex; the antineuritic factor will be spoken of as vitamin B (B_1); and the so-called antipellagra factor as vitamin G (B_2).

In these laboratories, a flattening of the growth curve was noted in experimental rats receiving an adequate diet with human milk as the only source of the vitamin B complex (5). The addition of autoclaved yeast stimulated some renewed growth, indicating that mother's milk in the amounts fed, contained insufficient vitamin G, or other factors present in yeast so treated, to sustain growth. An even greater response was produced by supplementing the diet with whole untreated yeast which bears evidence that both the antineuritic vitamin B and vitamin G, as well as well as other possible vitamin fractions present in yeast, may be limiting factors in the adequacy of mother's milk. By adding 10 grams of whole dried brewer's yeast daily to the diets of lactating women, the individual milks became more effective as a source of the vitamin B complex (11). Experimental rats, receiving milk from the mothers who had taken yeast, grew at an accelerated rate on less food. A clinical counterpart in the human infant has been reported by Hess (12) who gave whole brewer's yeast directly to infants; likewise there was a gain in weight which the author explains, "was due no doubt, in some cases to the stimulation of appetite, but in others there was a prompt response although the total intake of food remained the same." It is an indication that some factor in yeast which effects a more efficient utilization of food by the infant is the same as that conferred to the milk of mothers taking yeast.

The occurrence of borderline deficiencies in infants (1–4) makes important the quantitative determination of each of the vitamin fractions in milk. Cow's milk has been found to be richer in vitamin G than the antineuritic vitamin B (13–14). The following note on the vitamin G concentration of pooled breast milk is preliminary to more extensive studies on the potency of milk as it is influenced by vitamin additions to the human maternal dietary.

EXPERIMENTAL PROCEDURE. The method followed in determining the vitamin G content of human milk was essentially that developed by Bourquin and Sherman (16). A unit of vitamin G is taken as the amount of test substance necessary to cause an average weekly gain of 3 grams in standardized test rats over a period of 8 weeks, the animals having been depleted previously of any vitamin storage by retaining them on a vitamin G-free diet for a period of two weeks or more.

The basal vitamin G-free diet was fed *ad libitum* and consisted of 18 grams casein,[4] 68 grams dextrin,[5] 4 grams Osborne and Mendel salt mixture, 8 grams filtered butter fat, and 2 grams cod liver oil. Apart from

[4] The casein was treated with acidulated water (16), then extracted with 60 per cent alcohol (17) followed by washing with 95 per cent alcohol.

[5] An 80 per cent alcoholic extract of whole wheat equivalent to 50 grams of untreated wheat was dried upon 18.75 grams of the dextrin.

the basal diet, graded quantities of fresh pooled human milk[6] and 2 drops of wheat germ oil[7] were fed daily.

The positive control rats received the same diet as the experimental animals on milk with the exception that the source of vitamin G was alkaline autoclaved yeast instead of milk. Since yeasts vary widely in the potency of both vitamins B and G (18, 19), it is necessary to know the original potency of samples to be used in experimental studies as well as the amount of destruction of the various factors by different methods of treatment.

By preliminary tests, it was demonstrated that the yeast used in this study was rich in both factors. One sample was autoclaved for a period of from 3 to 6 hours at a pH of approximately 4.5 and another sample in an alkaline medium to note the degree of destruction of the vitamins by the various types of treatment. The results showed that the yeast that had been moistened with tenth normal NaOH and autoclaved for 6 hours at 15 pounds pressure (18) had a more complete destruction of vitamin B than did the samples autoclaved at a pH of approximately 4.5 for the same length of time. For this reason, the alkalized autoclaved yeast was used as a source of vitamin G for the positive control animals in this study. Such severe treatment of the yeast, however, caused some decrease of vitamin G also, as was shown by lessened rate of growth in the test animals. This is in accord with the findings of other investigators (20–22).

Results and discussion. The growth of the standardized test rats fed daily quantities of 3 cc., 5 cc., 10 cc., 15 cc., and 20 cc. of fresh pooled human milk as the only source of vitamin G in a diet otherwise adequate is given in chart 1. A group of animals on the vitamin G-free basal ration was observed as negative controls and another on the same diet plus 0.40 gram alkalized autoclaved yeast daily, served as the positive controls.

The results demonstrate that as the supply of milk was increased, the growth rate of the animals likewise increased. The mean increments in weight of the rats for the 8 weeks' experimental period on the various levels of milk were as follows: 3 cc. gave 19 $\pm$ 3.0 grams, 5 cc. gave 32 $\pm$ 2.6 grams, 10 cc. gave 41 $\pm$ 2.4 grams, 15 cc. gave 72 $\pm$ 3.7 grams, and 20 cc. gave 94 $\pm$ 1.2 grams. But even at the 20 cc. level, the highest amount of milk fed, the growth of the animals did not approximate that of the positive controls which made gains of 154 $\pm$ 1.2 grams.

The negative control rats showed varying degrees of vitamin G de-

[6] The mothers contributing milk for this study were American women coming from average homes. They chose their diet unrestrictedly except that no supplementary additions of yeast were permitted. Each produced enough milk to feed her own infant as well as furnish 20 to 25 ounces each day to the Mother's Milk Bureau of Detroit.

[7] An anhydrous ether extract of wheat germ.

ficiency. There was a definite stasis in growth within two to four weeks after the experimental diet was instituted. These negative controls de-

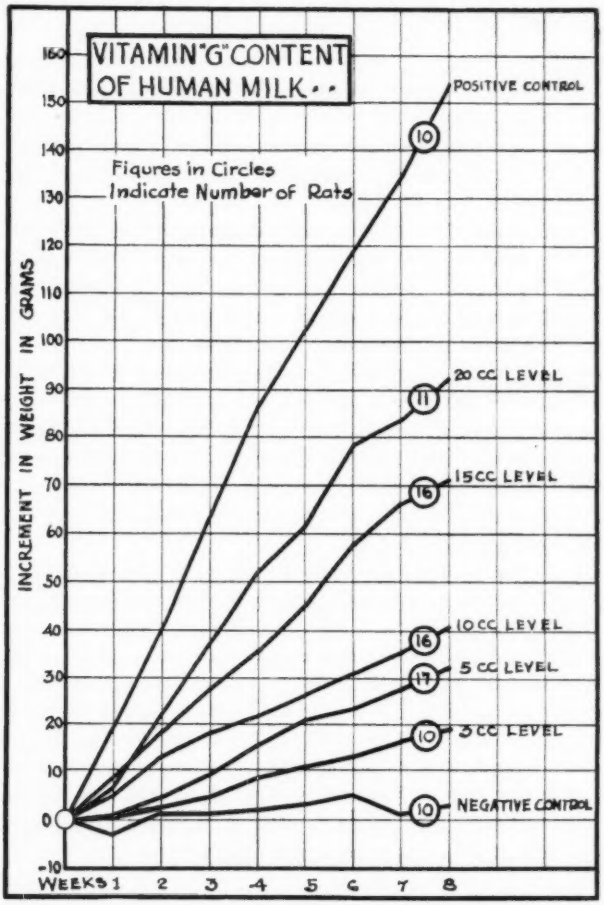


Chart 1. Illustrates the growth curves of the standardized test rats. The positive controls received the vitamin G-deficient basal diet plus 0.4 gram alkalized autoclaved yeast daily as the source of vitamin G; the negative controls received the basal diet without supplements; the experimental rats received adjuvants of 3 cc., 5 cc., 10 cc., 15 cc., and 20 cc. of fresh pooled breast milk daily as the sole source of vitamin G for a period of 8 weeks. Figures in the circles indicate the number of animals in each group from which the composite growth curves are computed.

veloped an unkempt appearance and the tails, ears, and medial portions of the legs became encrusted with a waxy, sebaceous deposit. Alopecia

was common, particularly around the eyes; pronounced pellagra-like lesions were not always present, but in all animals on a vitamin G-deficient diet there was an inflammatory condition of the forepaws and nose with evidence of abrasions.

In the present study, the unit rate (15) of growth of the rats was attained by feeding 3 cc. and 5 cc. quantities of milk per day per rat. Although these quantities of mixed human milk were sufficient to support some gain in body weight of the experimental rats, deficiency symptoms appeared in 70 per cent of the animals receiving 3 cc. of milk, and 59 per cent of those getting 5 cc. each day.

The final weights of the animals receiving smaller amounts of milk were more variable than those getting larger quantities. It is noted that for the daily intake of 3 cc. and 5 cc. of breast milk, the coefficients of variability were 28 and 25 respectively in contrast to 13, 18, and 6 for the 10 cc., 15 cc., and 20 cc. respectively.

The depletion of yet unidentified dietary essentials can not be cited as the causal factor of the greater variability among groups getting less milk, for, as expressed by Sherman (15, 19), these factors are conserved by the slow growth rate obtained when small quantities of the test vitamin are fed. Thus, in these observations on the lower levels of milk, the greater variability may be attributed to the lack of vitamin G and the accompanying lowered physical state.

In connection with this study, it is of interest to review the observations recorded by Guha (23) in which he reports that experimental rats that had reached a stasis in growth on a diet containing vitamin B in the form of a Fuller's earth preparation of wheat germ and vitamin G as an alkaline autoclaved marmite were stimulated to resume growth by the addition of cow's milk to the diet. In these laboratories, when breast milk was fed to standardized rats as the only source of the vitamin B complex in an otherwise complete diet, autoclaved yeast supplements stimulated a resumption in growth. It would appear then that the undetermined dietary factor described by Guha is present in breast milk and that it is not a limiting factor in the studies recorded herein.

SUMMARY

Under the conditions of these experiments, fresh mixed human milk in quantities from 3 cc. to 5 cc. daily as the only source of vitamin G in a diet otherwise complete, was sufficient to support the unit gain in weight of 3 grams weekly in young standardized test rats throughout the experimental period of 8 weeks. The greater number of animals, however, on these levels of milk showed deficiency symptoms. Quantities of 10 cc., 15 cc., and 20 cc. daily produced increasing gains in body weight, although none attained the rate of growth established by the positive controls.

If the above facts on breast milk produced by mothers on dietaries which are known to be adequate can be applied to infant feeding on the basis of comparative body weights, the quality of breast milk in some cases where the maternal diet is limited, either through choice or deprivation, may fall short of satisfying the vitamin G needs of the rapidly growing infant.

BIBLIOGRAPHY

(1) Hoobler, B. R. Journ. Amer. Med. Assoc, 1928, xci, 307.
(2) Dennett, R. H. Journ. Amer. Med. Assoc., 1929, xcii, 769.
(3) Daniels, A. L. Amer. Journ. Dis. Child., 1922, xxiii, 41.
(4) Bloxsom, A. P. Amer. Journ. Dis. Child., 1929, xxxvii, 1161.
(5) Macy, I. G., J. Outhouse, A. Graham and M. L. Long. Journ. Biol. Chem., 1927, lxxiii, 189.
(6) Outhouse, J., I. G. Macy, V. Brekke and A. Graham. Journ. Biol. Chem., 1927, lxxiii, 203.
(7) Gibson, R. B. and I. Concepcion. Philippine Journ. Sci., Sect. B., 1916, xi, 119.
(8) Osborne, T. B. and L. B. Mendel. Carnegie Inst. Washington Year Book, 1920, xix, 389.
(9) Poulsson, E. Norsk. Mag. f. Laegevidenskaben, 1923, xx, 35; Chem. Abst., 1924, xviii, 410.
(10) Gerstenberger, H. J., J. I. Hartman and D. N. Smith. Calif. West. Med., 1927, xvii, 40.
(11) McCosh, S. S., I. G. Macy and H. A. Hunscher. Journ. Biol. Chem., 1931, xc, 1.
(12) Hess, A. F. Amer. Journ. Dis. Child., 1917, xiii, 98.
(13) Sherman, H. C. and J. H. Axtmayer. Journ. Biol. Chem., 1927, lxxv, 207.
(14) Hunt, C. H. and W. E. Krauss. Journ. Biol. Chem., 1928, lxxix, 733.
(15) Bourquin, A. and H. C. Sherman. Journ. Amer. Chem. Soc., 1931, liii, 3501.
(16) McCollum, E. V. and M. Davis. Journ. Biol. Chem., 1915, xxiii, 231.
(17) Sherman, H. C. and A. Spohn. Journ. Amer. Chem. Soc., 1923, xlv, 2719.
(18) Quinn, E. J., F. B. Whalen and J. G. Hartley. Journ. Nutr. 1930, iii, 257.
(19) Chase, E. F. and H. C. Sherman. Journ. Amer. Chem. Soc., 1931, liii, 3506.
(20) Williams, R. R., R. E. Waterman and S. Gurin. Journ. Biol. Chem., 1929, lxxxiii, 321.
(21) Chick, H. and M. H. Roscoe. Biochem. Journ. 1930, xxiv, 105.
(22) Chick, H. and A. M. Copping. Biochem. Journ. 1930, xxiv, 932.
(23) Guha, B. C. Biochem. Journ. 1931, xxv, 960.

THE VITAL CAPACITY OF THE LUNGS AT LOW BAROMETIC PRESSURE.

EDWARD C. SCHNEIDER

From the Department of Biology, Wesleyan University, Middletown, Connecticut

Received for publication February 11, 1932

Authorities have for a long time agreed that a decrease in the vital capacity of the lungs takes place when one is first exposed to a low barometric pressure. Of these the earliest record is that of Paul Bert (1878) who noted a diminution of as much as 50 per cent in the vital capacity at the summit of Monte Rosa.

The change in vital capacity seems to be without practical importance in the adaptations for obtaining oxygen and may be, therefore, of only theoretical interest. The writer having recently had occasion to review the literature on the subject was stimulated to delve into experimental data he has accumulated from time to time over a period of 19 years. Almost every group of physiologists that undertakes an experimental expedition into the mountains for the study of acclimatization sooner or later makes observations on the vital capacity. The last to report on a series of tests was Grollman, in 1930, who observed a decrease of 11.2 per cent in himself while on Pike's Peak.

Various theories have been advanced to account for the decrease. Zuntz and his co-workers (1906) attributed it to an expansion of intestinal gases; Durig (1909) looked upon fatigue of the respiratory muscles as the explanation; Fuchs (1908) regarded it as a result of increased muscular tone due to the low temperatures at which the subjects lived; and Mosso (1889) argued that the blood collects in the lungs during inspiration in excess of the amount expelled by the following expiration, thus leaving less space for air. Since most of the bodily changes observed at high altitudes can be directly or indirectly attributed to the relative shortage of oxygen there experienced, it is here our purpose to inquire whether the decrease in vital capacity may not be, at least in part, due to anoxemia.

Observations in a low pressure chamber. During a period of two years data on the vital capacity were accumulated from observations made on 24 men while exposed to low barometric pressures in a low pressure chamber. Only a part of the data is given here. It was our custom to lower

the pressure by stages and to maintain a constant pressure at each step for from 10 to 15 minutes. We ordinarily used simulated altitudes of from 18,000 or 20,000 feet and occasionally as high as 25,000 feet. We invariably secured some reduction in the vital capacity, the average decrease at 20,000 feet was 350 cc., approximately 8 per cent; while the maximum for one man at that altitude was 1080 cc., or a 25 per cent reduction. There was considerable individual variation in the amount of reduction in atmospheric pressure required to induce a decrease in vital capacity. No one gave a definite response at less than a simulated altitude of 10,000 feet, while the break for most of our subjects came at altitudes of from 12,000 to 15,000 feet. The breaking point of two men is shown in table 1. In these cases the step upward in simulated altitude was by units of 2000 feet each. The run was up to 20,000 feet and then by steps back to sea-level. One of these men experienced the change at about 14,000 and the other at around 18,000 feet.

TABLE 1

A study to determine the "point of break" in the vital capacity of 2 subjects at simulated altitudes

Vital capacity in liters

SEA LEVEL	6,000 FEET	8,000 FEET	10,000 FEET	12,000 FEET	14,000 FEET	16,000 FEET	18,000 FEET	20,000 FEET	18,000 FEET	16,000 FEET	14,000 FEET	12 000 FEET	10,000 FEET	SEA LEVEL
4.85	4.85	4.85	4.85	4.86	4.80	4.86	4.73	4.55	4.70	4.75	4.81	4.80	4.91	4 89
4.72	4.76	4.85	4.79	4.75	4.62	4.55	4.35	4.35	4.40	4.57	4.65	4.73	4.74	4.89

Table 2 presents three series of observations made in a study of the effects of the administration of pure oxygen. There were seven men who served as subjects, the data for only three of these are given in the table. During exposure to lowered barometric pressure without access to extra oxygen, all of the men showed a reduction in vital capacity. In the second series of runs each man received a large and steady flow of oxygen throughout the entire period of exposure to the reduced barometric pressure. The oxygen was delivered through a rubber tube that was held in the mouth like a pipe stem. The average decrease in vital capacity for the group of 7 cases at the simulated altitude of 20,000 feet was 360 cc. without oxygen and 130 cc. when oxygen was supplied throughout the run. It is evident, therefore, that oxygen may in large part prevent the decrease in vital capacity. Only one man, however, was completely saved from this effect by the oxygen (see T. V. in table 2).

In the third series of runs of which three examples are given in table 2, the men went without an extra supply of oxygen until they reached the simulated altitude of 20,000 feet. After 10 minutes of exposure to this

pressure, they were given oxygen in the usual way. The average decrease in vital capacity for the group of seven men was 510 cc. when 20,000 feet was reached. The breathing of the extra oxygen raised the average capacity from 3940 cc. to 4230 cc., which was only 220 cc. below the normal, 4450 cc., for the group. The return to normal was complete in three of the men, while a fourth gained nothing by breathing the extra supply of oxygen.

The method of administering oxygen was wasteful and undoubtedly did not raise the alveolar oxygen tension of all the subjects in equal degree.

TABLE 2

Vital capacity without O_2 during exposure to low barometric pressure, while taking O_2 throughout the run, and as a result of taking O_2 at the lowest pressure

Vital capacity in liters

SUBJECT	STIMULATED ALTITUDE						
	0 feet	10,000 feet	15,000 feet	18,000 feet	20,000 feet	22,000 feet	25,000 feet
Without oxygen							
P. B.	4.50	4.32	4.20	4.14	3.95		
S. F.	4.42	4.40	4.40	4.05	3.85		
T. V.	4.35	4.30	4.30	4.25	4.15		
Taking O_2 from the start							
P. B.	4.40	4.36	4.24	4.40	4.33	4.32	4.19
S. F.	4.38	4.30	4.15	4.13	4.11	4.10	4.01
T. V.	4.39	4.31	4.29	4.21	4.34	4.30	4.33
Without O_2 and then administration of O_2 at 20,000 feet, lowest pressure							
						20,000 feet	
P. B.	4.50	4.32	4.15	4.00	3.95	*4.05*	
S. F.	4.32	4.30	4.43	4.00	3.98	*4.21*	
T. V.	4.75	4.68	4.48	4.42	4.40	*4.80*	

It is unfortunate that we made no analyses of the alveolar air for oxygen. Our results indicate that the decrease in vital capacity during exposure to a low barometric pressure is due to anoxemia, and that it may be wholly, or at least in part, prevented by the administration of oxygen.

That the decrease in vital capacity is not due to the expansion of intestinal gases was indicated in the "come back" of our cases when oxygen was administered. Breathing an extra supply of oxygen would in no way affect the gas volume in the intestine. We measured the circumference of the abdomen of certain of our subjects and found some had a noticeable expansion without a marked reduction in vital capacity, while others had a

marked reduction in capacity without any external evidence of the presence of intestinal gases.

Observations on Pike's Peak. During three expeditions to the summit of the Peak (altitude 14,110 feet) the change in vital capacity was studied on nine men; two of the men were under observation during two, and a third during three, sojourns on the mountain. The data for two of the expeditions are summarized in table 3. It was our custom to allow three trials each time a man was tested. The data in the table give the largest volume obtained each day.

The vital capacity of every man was at first distinctly decreased by the ascent of Pike's Peak, this decrease ranging from 6.7 to 15.3 per cent.

TABLE 3

Vital capacity, in cubic centimeters, as obtained on Pike's Peak

SUBJECT	R. A.	H. G.	L. H.	E. S. 1	D. S.	G. C.	W. R.	E. S. 2
Colo. Springs								
Smallest	5,880	3,920	5,300	4,250	3,800	4,510	4,200	4,480
Largest	6,020	3,950	5,490	4,450	3,970	4,600	4,310	4,510
Pike's Peak								
Day 1	5,100	3,550	5,050	4,020	3,600	4,200	3,990	4,160
2	5,480	3,530	4,990	4,000	3,530	4,310	4,100	4,260
3	5,430	3,700	5,150	4,100	3,600	4,480	4,140	4,370
4	5,600	3,710	4,950	4,200	3,650	4,510	4,170	4,440
5	5,550	3,675	5,230	4,125	3,710	4,500	4,200	4,400
6			5,200	4,100	3,700	4,380	—	—
7			5,230	4,025	3,600	4,360	4,070	—
8			5,220	4,275	3,790	4,530	4,300	4,440
9			5,210	—	3,690	4,515	4,190	4,440
10			—	—	—			
11			5,190	4,275	—			
11			—	—	—			
12			5,190	4,275	—			
13			5,300	4,250	3,680			

As a rule the maximum decrease was obtained during the first day of residence on the mountain, but in several of the men it occurred on the second. The ascent of the mountain was ordinarily made by cog-railway train, but four of our subjects climbed the mountain afoot. It was the men of the latter group who showed the largest reduction in the vital capacity, 10.6 to 15.3 per cent (see R. A. and H. G., table 3).

In all of our subjects the vital capacity sooner or later showed a marked tendency to return to normal. This was usually in evidence by the second day of residence, or at least by the third day. In two of the men, who walked up the mountain, the beginning of the recovery was delayed five

days; while in one, L. H., who ascended passively on the railway train, there was an equal delay in two expeditions. Among the men who remained from 9 to 13 days on the mountain the return to normal in vital capacity was almost, but never quite, complete. The data in table 3 show that the return for L. H., E. S., G. C. and W. R. was sufficient to bring it within the limits obtained at the low altitude; but that W. R. alone, while on Pike's Peak, had a vital capacity equal to his maximum determination at Colorado Springs, the low altitude.

In three journeys to the summit the vital capacity of E. S. was reduced by 7.1, 9.3, and 10.1 per cent respectively; and during each of them the recovery was approximately the same. In two trips D. S. gave reductions of 11.7 and 11.1 per cent and L. H. of 6.7 and 8 per cent respectively. These observations indicate that the decrease in barometric pressure reduces the vital capacity of the lungs to quite the same degree with every ascent of the mountain.

Observations on one man, who resided [illegible]mit of Pike's Peak from early in May to November, indicate that with complete acclimatization the vital capacity at the high altitude comes to be the same as at low altitudes. Repeated tests were made on this man while he was on the summit of the mountain during a period of five days in October, and showed his vital capacity to range between 3950 and [illegible]cc. The following winter, while he was in Colorado Springs, the determinations gave a range between 3970 and 4220 cc.

The effect of oxygen while on Pike's Peak. On two days, about the middle of a 14 day sojourn on the mountain, oxygen was administered for a period of 15 minutes to three of our subjects. In each of the six trials the breathing of oxygen resulted in some increase in the vital capacity, but in no instance did it restore the capacity to the maximum of Colorado Springs. The best result obtained for each man with the use of oxygen was as follows: L.H. was raised from 5120 to 5300 cc.; E.S., from 4000 to 4250 cc.; and D.S., from 3620 to 3800 cc. In view of the fact that the funnel method of administering oxygen was employed, it is questionable whether we succeeded in raising the alveolar oxygen pressure in the lungs to that found at the low altitude. Hence we may conclude that the reduction in the vital capacity of the lungs, which is experienced at high altitudes in the mountains, is a result of anoxemia.

The effect of atmospheric cold. Since Fuchs made the suggestion that the decrease in vital capacity at high altitudes is due to an increase in muscle-tone caused by low atmospheric temperature, we decided to test out this point. On two different days two of the men remained out of doors, quite inactive, for upwards of an hour. During this time the vital capacity was repeatedly determined by passing the pipe of the spirometer to them through a crack in a door. In no instance was the vital capacity of these

men lessened by their exposure to temperatures of 16° and 18°F. The other data we have reported were obtained in a room at temperatures ranging from 67° to 72°F.

The cause of the decrease in vital capacity. Our observations in the low pressure chamber and on Pike's Peak seem to refute some of the theories offered to explain the reduction in vital capacity. They tend to eliminate the theories which attribute the change to an expansion of intestinal gases or to a tonic effect of low temperatures. They tend to cast doubt on the theory that attributes the reduction to fatigue of the respiratory muscles. It is true that men who walked up Pike's Peak, on the whole, experienced the greatest reduction; but the differences were not proportionate to the fatigue. One of those men, H.G. table 3, was extremely exhausted and prostrated, yet he had a reduction of only 10.6 per cent; while R.A., who arrived feeling very well, showed a decrease of 15.3 per cent in his vital capacity. R. A., 15 minutes arriving on summit, had a vital capacity of 5600 cc.; two hours later fallen to 5450 cc.; and five hours after the ascent, it was only 5100 cc., which would seem to eliminate fatigue as a cause.

The work of Drinker, Peabody and Blumgart (1922) on the effect of pulmonary congestion on the vital capacity of the lungs in heart disease shows that an engorgement of the pulmonary blood vessels may take up alveolar space which air could occupy under normal conditions. In 1903 Bartlett presented experiments that showed that the pulmonary vessels, when exposed to diminished air pressure, dilate and thereby produce congestion in the lesser circulation. This he attributed to an equalization of the atmospheric and the intrathoracic pressures. Schubert (1930) similarly believes that in rarefied air the elastic force of the alveoli has less to oppose, consequently the alveolar walls relax and their lumen becomes smaller. Along with this the capillaries enlarge and passive congestion follows. It seems unlikely, in view of the recovery in vital capacity that we obtained during oxygen inhalation, that Bartlett's and Schubert's explanations can be applied to our problem. Grollman (1930) found that the heart output per minute in two subjects while on Pike's Peak increased steadily for four or five days, reaching a maximum of about 40 per cent above its sea-level value and then gradually declined to its normal level. This is the inverse of the series of changes some of our subjects experienced in the vital capacity of their lungs. Conceivably reserve capillary paths may be brought into requisition during the early stages of acclimatization. Vannotti (1931) was able to identify particular capillary loops in the skin and in these definite dilatation was observed at high altitudes. It may be that the anoxemia experienced under low barometric pressure causes a relaxation or loss of tonus of the pulmonary capillaries, and that this in turn is followed by capillary engorgement; or that sluggish and empty capil-

lary areas may be brought into active circulation. The beneficial effect we obtained by administering oxygen would very likely be on the capillary endothelium which was thus enabled to return to or toward its natural tonus.

SUMMARY

A decrease in the vital capacity of the lungs was obtained by subjecting men to a reduced barometric pressure in a low pressure chamber. Individual differences occurred; no one experienced the change at less than a simulated altitude of 10,000 feet; while in the majority of cases this occurred at simulated altitudes of from 12,000 to 15,000 feet. The use of an extra supply of oxygen prevented the effect in part in some men and wholly in others.

By measuring the circumference of the abdomen, it was shown that the decrease in vital capacity is not the result of the expansion of intestinal gases.

On Pike's Peak, altitude 14,110 feet, the vital capacity was decreased during the first day or two by from 6.7 to 15.3 per cent in nine men. Later all men showed at least a partial return to normal. With acclimatization the recovery was complete. During the period of reduced vital capacity the administration of oxygen caused a partial recovery.

A low atmospheric temperature was shown not to be the cause of the decrease.

It is suggested that the reduction in vital capacity is due to an engorgement of pulmonary blood vessels, that reserve capillary paths are brought into requisition, and that possibly there is a relaxation of pulmonary capillaries as a result of anoxemia. The beneficial effect obtained by the administration of oxygen may be found to be due to restored capillary tonus.

BIBLIOGRAPHY

BARTLETT, F. H. 1903. This Journal, x, 149.

BERT, P. 1878. La pression barométrique. Paris, 716.

DRINKER, C. K., F. W. PEABODY AND H. L. BLUMGART. 1922. Journ. Exper. Med., xxv, 77.

DURIG, A. 1909. Physiologische Ergebnisse der im Jahr 1906 durchgeführten Monte Rosa Expedition. Wien.

FUCHS, R. F. 1908. Sitzungsb. Physick. Med. Soz., Erlangen, xl, 238.

GROLLMAN, A. 1930. This Journal, xciii, 19.

MOSSO, A. 1889. Life of man on the high Alps. London, 153.

SCHUBERT, G. 1930. Pflüger's Arch., cciv, 260.

VANNOTTI, A. 1931. Klin. Wochenschr., x, 253.

ZUNTZ, N., A. LOEWY, F. MÜLLER AND W. CASPARI. 1906. Höhenklima und Bergwanderungen. Berlin.

SOME PHASES OF LIVER AND GALL-BLADDER FUNCTION

Experiments on Rabbits with Certain Organic Compounds, Particularly Dyes

BÉLA HALPERT and MILTON THEO. HANKE

From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago; and the Department of Surgery, Yale University School of Medicine

Received for publication February 12, 1932

A substance that is excreted into the bile, that will accumulate in the gall bladder, that is opaque to Roentgen rays and that possesses bactericidal properties would probably be of value in the diagnosis and treatment of certain diseases of the gall bladder. The procedure used in our former experiments provides a simple direct method for determining whether or not a chemical compound is excreted into the bile and if it accumulates in the gall bladder (in animals).

In this preliminary investigation we have studied four dyes, namely, tetraiodophenolphthalein and the closely related flourescein derivatives: eosin, erythrosin and rose bengal, as well as sodium salicylate and the sodium salt of diiodosalicylic acid.[1]

Experimental procedures. *Surgical procedure.* The method was identical with that previously described (1).

Mode of administration. The compounds studied were either injected intravenously or administered orally (by stomach tube).

Analytical methods. The fluorescein derivatives, *eosin*, *erythrosin* and *rose bengal*, are so intensely colored that a special chemical method is not required for their determination. The excreted bile and the bile in the gall bladder are colored intensely red as soon as the excretion of the dye has begun. It usually suffices, therefore, to dilute the bile accurately to some convenient concentration and compare this solution, in a colorimeter, with a standard solution of the dye. The inherent color of the bile is a negligible factor until the concentration of the dye has become so minute (1:10,000) that it too becomes negligible.

Salicylic acid was determined as follows: Bile or urine—10 cc.—is mixed with 90 cc. of a saturated solution of sodium bicarbonate. The

[1] A brief summary of the results of some of these experiments was read (by title) at the Forty-fifth Session of the American Association of Anatomists (4) and at the XIIIth International Physiological Congress (6).

resulting liquid is extracted three times with ether using 200 cc. for each extraction. The ether extract is discarded. Neutralize the alkaline solution with 7N sulfuric acid (congo red paper) and add 2 cc. of sulfuric acid in excess. The acid liquid is extracted three times with ether using 200 cc. for each extraction. The combined ether extracts, which contain the salicylic acid, are evaporated in a glass dish at room temperature by means of a current of dry air. A saturated solution of sodium bicarbonate —1.0 to 1.5 cc.—is added to the residue in the glass dish. Transfer, with water, to a 20 cc. volumetric flask and dilute to the mark. The salicylic acid is determined in 10 cc. of this liquid by the Folin and Ciocalteu modification of the Millon reaction (5). The color is compared with that produced by 5 mgm. of salicylic acid in 10 cc. of a solution of an alkalinity equivalent to that of the test solution.

Diiodosalicylic acid and *tetra iodophenolphthalein* were not determined directly. Bile and urine were analyzed for iodine (method below) and the amounts of the diiodosalicylic acid or of the tetra iodophenolphthalein determined by calculation.

Method for determining iodine in bile or urine. The bile or urine (1 cc.) is measured into a silver crucible and evaporated to dryness on the steam bath. Place 20 grams of potassium hydroxide in another silver crucible and heat until all the water is driven off. Pour the molten KOH into the crucible containing the dried bile or urine and heat carefully until the melt is a clear, straw colored liquid. The fusion usually takes 3 to 4 minutes. Allow the material to cool and transfer quantitatively, with distilled water, to a 500 cc. Erlenmeyer flask. The transfer should involve the use of about 125 cc. of water. Acidify with 40 cc. of 85 per cent phosphoric acid, add 50 cc. of saturated chlorine water, and boil. Considerable water must be vaporized before the liquid is free from Cl_2. Additional water may have to be added. The absence of Cl_2 can be determined by the usual starch KI method. *Cool*, add a pinch of KI and starch solution (about 1 cc.), and titrate immediately with 0.02 N sodium thiosulfate.

Experiments with eosin (*a*), *erythrosin* (*b*), *and rose bengal* (*c*). It is mentioned by Graham and his collaborators that the above halogenated derivatives of fluorescein produce cholecystograms (2). Staining of the tissues, however, precluded their clinical use. The following are data on the mechanism of their excretion.

a. Eosin (tetrabromfluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight, appeared in the bile in three to five minutes; it reached the highest concentration in the bile collected from the ductus choledochus within 24 to 42 minutes. This maximum concentration—1:51 to 1:122—was approximately maintained for from 28 to 53 minutes. Values ranging between 1:2,900 and 1:14,000 were obtained 6 hours after the injection. The bile removed

from the gall bladder at the same time, i.e., 6 hours after the injection, contained eosin in a higher concentration than the last sample of bile obtained from the ductus choledochus (table 1). In each animal the concentration of eosin in the bile removed from the gall bladder, however, was always less than the highest concentration reached in the bile collected from the ductus choledochus.

b. Erythrosin (tetraiodofluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight, appeared in the bile within 3 to 4 minutes; it reached the highest concentration in the bile collected from the ductus choledochus within 23 to 33 minutes. This maximum concentration—1:80 to 1:200— was approximately maintained for from 14 to 67 minutes. Values ranging between 1:900 and 1:20,000 were obtained 6 hours after the injection. The bile removed from the gall bladder at the same time, i.e., 6 hours after the injection, contained from 10 to 7 hundred times as much erythrosin as that contained in the last sample of bile obtained from the ductus choledochus (table 1). The concentration of erythrosin in the bile removed from the

TABLE 1

Summary of data on the excretion of eosin, erythrosin, and rose bengal by the biliary system of the rabbit following their intravenous administration in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight

RABBIT	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM D. CHOLEDOCHUS			DURATION OF EXPERIMENT	AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution	Approximately maintained for			From D. choledochus	From gall bladder	Ratio of concentration approximate
				Eosin					
	minutes	*minutes*		*minutes*	*hours*	*cc.*			
I-1	4	24	1:51	28	6	6.3	1:4,700	1:90	1:52
I-3	3	29	1:100	53	6	4.3	1:2,900	1:2,000	1:1.4
I-2	5	42	1:122	42	6	3.7	1:11,600	1:3,060	1:3.7
				Erythrosin					
I-3	4	25	1:80	17	6	7.5	1:20,000	1:28	1:714
I-2	3	23	1:100	14	6	11.4	1:11,500	1:720	1:15.8
I-1	4	33	1:200	67	6	2.7	1:900	1:90	1:10
				Rose bengal					
I-3	5	55	1:142	75	6	3.5	1:1,180	1:750	1:1.5
I-1	7	65	1:144	15	3	5	1:960	—	—
I-2	6	32	1:192	35	2	4.5	1:340	1:252	1:1.3

gall bladder at the end of the sixth hour following the injection was higher than the highest concentration that it reached in the bile collected from the ductus choledochus. This was true in two out of three animals.

In one rabbit 40 mgm. (i.e., 2 cc. of a 2 per cent solution) of erythrosin per kgm. of body weight were injected intravenously. The results closely resembled those obtained by the injection of half this amount, i.e., the dye appeared in the bile about 3 minutes following the injection; it reached the highest concentration in the bile collected from the ductus choledochus within 30 minutes; this maximum concentration—1:63—was approximately maintained for about 40 minutes; 3 hours following the injection the concentration of erythrosin in the bile collected from the ductus choledochus was 1:2000, in that removed from the gall bladder 1:45.

Erythrosin could not be detected in the bile collected from the ductus choledochus or in that removed from the gall bladder 24 hours following the administration to one rabbit intravenously and to another by stomach tube of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) of erythrosin per kgm. of body weight.

c. Rose bengal (tetraiodotetrachlorfluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight appeared in the bile in 5 to 7 minutes; it reached the highest concentration in the bile collected from the ductus choledochus in 32 to 65 minutes. This maximum concentration—1:42 to 1:192—was approximately maintained for from 15 to 75 minutes. The values obtained in 3 animals, 2, 3 and 6 hours following the injection were 1:340, 1:960, and 1:1,180 respectively. The bile removed from the gall bladder at the same time contained rose bengal in a concentration of 1:252 two hours, and 1:750 six hours following the injection (table 1). Thus the concentration of rose bengal 2 and 6 hours following the administration was higher in the bile removed from the gall bladder than in the sample of bile collected from the ductus choledochus. This concentration, however, was less than the highest concentration reached in the bile collected from the ductus choledochus.

Rose bengal cannot be determined in the bile collected from the ductus choledochus 24 hours after the intravenous administration of the dye. Rose bengal is, however, still present in the gall bladder at the end of this time interval. The gall bladder bile of 3 rabbits that had received 40 mgm. of rose bengal per kgm. of body weight contained the dye in concentrations of 1:46, 1:73 and 1:118 respectively; the gall bladder bile of 3 rabbits that received 60 mgm. of rose bengal per kgm. of body weight contained the dye in concentrations of 1:60, 1:111 and 1:230 respectively, 24 hours after injection.

Rose bengal cannot be determined in gall-bladder bile 48 hours after the dye has been injected.

Excretion of the dyes into the bile continued for several hours even when the dyes were administered intravenously. This would suggest that at least a portion of the dye remained in the blood stream. The presence of the dyes could not, however, be determined by a direct examination of the blood. Better results were obtained by an indirect method. The procedure adopted was that of removing the aqueous humor twice in succession from one eye, using the other eye as a control. These experiments were carried out upon the suggestion and with the coöperation of Dr. Peter C. Kronfeld of the Department of Ophthalmology, University of Chicago. None of these dyes (eosin, erythrosin, rose bengal) appeared spontaneously in the aqueous humor removed one and two hours following the intravenous administration of the dyes in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight. A second sample of the aqueous humor, on the other hand, removed 15 minutes after the first, invariably contained the dye (3).

Experiments with sodium salicylate (*a*), *and sodium salt of diiodosalicylic acid* (*b*). In view of the differences of opinion regarding the action of salicylates on the biliary system as cholagogues and as bactericides, it seemed desirable to investigate the excretion of the above two representatives of the group.

a. Sodium salicylate was given intravenously in doses of 40 mgm. (i.e., 4 cc. of 1 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 3 hours in one rabbit and 4 in another. The flow of bile averaged 5 and 9 cc. per hour. Neither these samples, nor the bile removed from the gall bladder at the end of the experiment, contained salicylates in determinable quantities (4).

b. Sodium salt of diiodosalicylic acid was given intravenously in doses of 40 gm. (i.e., 4 cc. of a 1 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 2 hours in one rabbit and 3 in another. The flow of bile averaged about 5.5 and 10 cc. per hour. Iodine, and therefore diiodosalicylic acid, was not present in determinable quantities in any of the samples of bile collected from the ductus choledochus or in that removed from the gall bladder at the end of the experiment.

Experiments with sodium salt of tetraiodophenolphthalein. With the object of securing quantitative data on the mechanism of the excretion with this dye, which is most extensively used for visualizing the gall bladder, three sets of experiments were performed. In the first series, *a*, sodium salt of tetraiodophenolphthalein (Iodeikon—Mallinckrodt) was given intravenously in doses of 20, 40 and 60 mgm. (i.e., 1, 2 and 3 cc. of a 2 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 6 hours. In the second series, *b*, iodeikon was injected intravenously in doses of 20 mgm. (i.e., 1 cc. of a 2

per cent solution) per kgm. of body weight and the operation performed at certain stipulated intervals between the 24th and 96th hours following the administration. In the third series, *c*, the same amount of iodeikon,

TABLE 2

Summary of data on the excretion of iodeikon by the biliary system of the rabbit following intravenous administration of 20, 40, and 60 mgm. (i.e., 1, 2, and 3 cc. of a 2 per cent solution) respectively per kgm. of body weight

RABBIT	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM D. CHOLEDOCHUS			DURATION OF EXPERIMENT	AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution	Approximately maintained for			From D. choledochus	From gall bladder	Ratio of concentration approximate
	minutes	*minutes*		*minutes*	*hours*	*cc.*			
I-10	20	39	1:136	30	6	2	1:747	1:927	1.2:1
I-8	7	40	1:209	40	6	2.2	1:1,215	1:1,195	1:1
I-7	8	35	1:133	60	6	6.6	1:5,160	—	—
I-6	15	60	1:143	30	6	2.2	1:554	1:452	1:1.2
I-9	5	55	1:31	60	6	3.1	1:92	1:18	1:5.1
I-11	16	110	1:78	75	6	2	1:420	—	—

TABLE 3

Concentration of the dye in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the rabbit 24, 48, 72, and 96 hours following intravenous administration of 20 mgm. (i.e., 1 cc. of a 2 per cent solution) of iodeikon per kgm. of body weight

RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE	RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE
24-hour group				72-hour group			
I-1	1:17,000	1:1,615	None	I-9	None	1:2,375	None
I-2	1:2,830	1:3,050	None	I-8	None	1:5,370	—
I-4	1:17,000	1:5,680	None	I-7	None	1:7,880	None
48-hour group				96-hour group			
I-5	None	1:5,100	None	I-10	None	1:5,100	None
I-3	1:12,800	1:6,010	None	I-12	None	1:11,900	—
I-6	None	1:7,750	None	I-11	None	None	—

(i.e., 1 cc. of a 2 per cent solution) per kgm. of body weight, was given by stomach tube and the operation performed 24, 48, 72 and 96 hours later.

The results obtained are charted in tables 2, 3 and 4, and may be sum-

marized briefly as follows: *a.* Iodeikon injected intravenously in doses of 20, 40 and 60 mgm. (i.e., 1, 2 and 3 cc. of a 2 per cent solution) per kgm. of body weight, led to the appearance of iodine in the bile in 5 to 20 minutes; it reached the highest concentration in the bile collected from the ductus choledochus in 35 to 110 minutes. The maximum concentration of the dye (as calculated from the iodine content)—1:31 to 1:209—was approximately maintained for from 30 to 75 minutes. Values ranging between 1:92 and 1:5,160 were obtained 6 hours after the injection. The bile removed from the gall bladder at the same time, i.e., 6 hours after the injection, contained about 1 to 5 times as much iodeikon as that contained in the last sample of bile obtained from the ductus choledochus (table 2). *b.* The dye was present in the bile collected from the ductus

TABLE 4

Concentration of the dye in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the rabbit 24, 48, 72, and 96 hours following oral administration of 20 mgm. (i.e., 1 cc. of a 2 per cent solution) of iodeikon per kgm. of body weight

RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE	RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE
24-hour group				72-hour group			
F-5	1:17,000	1:8,170	None	F-4	None	1:1,700	None
F-7	None	1:9,000	None	F-8	None	1:17,000	—
R-6	None	1:10,200	None	F-9	None	None	—
48-hour group				96-hour group			
F-1	1:17,000	1:1,510	None	F-10	None	None	None
F-2	1:17,000	1:2,550	None	F-11	None	None	None
F-3	None	1:6,150	None	F-12	None	—	None

choledochus in concentrations of between 1:2,830 and 1:17,000, and in the gall bladder in concentrations of 1:1,615 to 1:7,750, 24 and 48 hours following intravenous administration of iodeikon in doses of 20 mgm. per kgm. of body weight. Iodeikon could not be detected in the bile collected from the ductus choledochus 72 and 96 hours following administration; the gall bladder on the other hand contained iodeikon in concentrations from 1:2,375 to 1:11,900 (table 3). *c.* The dye was detected in the bile collected from the ductus choledochus in 3 of the 6 rabbits in a concentration of 1:17,000, and in the gall bladder in concentrations that varied between 1:1,510 and 1:10,200, 24 and 48 hours following oral administration of iodeikon in doses of 20 mgm. per kgm. of body weight. Iodeikon was not found in the bile collected from the ductus choledochus 72 and 96 hours following the administration; but the gall bladder con-

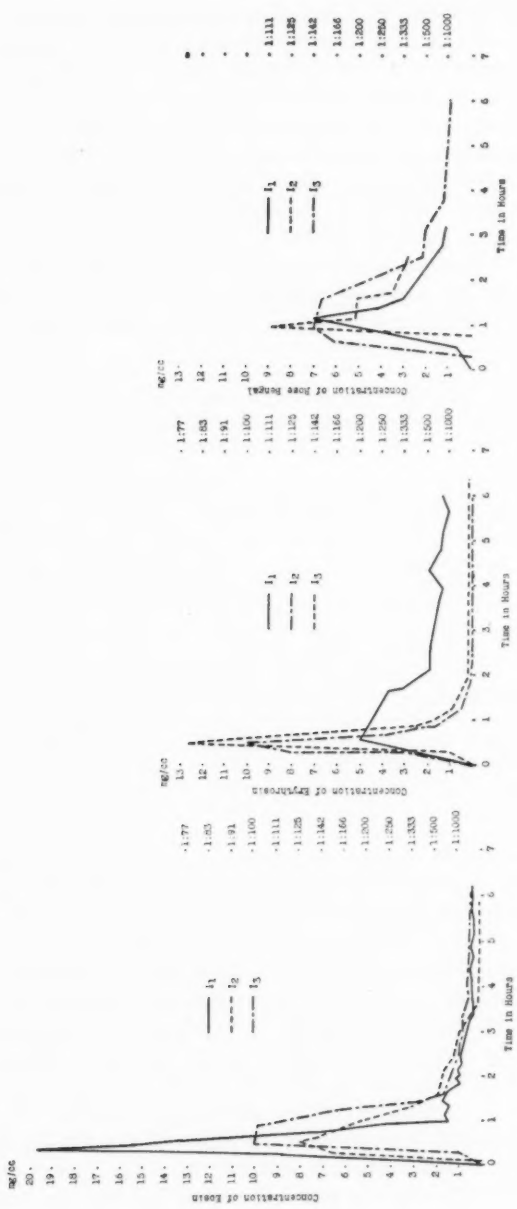


Fig. 1

Fig. 2

Fig. 3

tained iodeikon in 2 of the 6 rabbits in concentrations of 1:1,700 and 1:17,000.

COMMENT. All three of the halogenated derivatives of fluorescein studied appeared in the bile within a few minutes following their intravenous administration in doses of 20 mgm. per kgm. of body weight and invariably remained in the bloodstream for at least two hours. The curve indicating the dye content of the bile collected from the ductus choledochus was a regular one: a sharp rise was followed by a sudden decline (figs. 1, 2 and 3). In all instances the concentration of the dye was higher in the bile from the gall bladder than in the bile collected from the ductus choledochus 6 hours after the intravenous administration of eosin, erythrosin or rose bengal in spite of the fact that the gall bladder was never empty to begin with and that during this entire period of the experiment bile was passing through the cannulated ductus choledochus at a rate of 2.7 to 11.4 cc. per hour. Of these three dyes only erythrosin showed a higher concentration in the bile from the gall bladder than the maximum concentration ever reached in the bile collected from the ductus choledochus (table 1). Twenty-four hours following intravenous administration of rose bengal in doses of 40 and 60 mgm. per kgm. of body weight, the dye appeared in the bile removed from the gall bladder in concentrations between 1:46 and 1:230, and none in the bile collected from the ductus choledochus.

The experiments with sodium salicylate and the sodium salt of diiodosalicylic acid brought out the remarkable fact that these substances do not appear in the bile in determinable quantities after their intravenous administration in doses of 40 mgm. per kgm. of body weight.

SUMMARY

Eosin, erythrosin, or rose bengal injected intravenously into rabbits, in doses of 20 mgm. per kgm. of body weight, appeared in the bile within a few minutes. The bile collected from the ductus choledochus showed a sharp rise to the highest concentration—1:51 to 1:200—followed by a sudden decline. In all instances the concentration of the dye in the bile collected from the ductus choledochus 6 hours after the injection was less than in the bile simultaneously removed from the gall bladder, in spite of the fact that during the entire period of the experiment bile was passing through the cannulated ductus choledochus. Rose bengal was present in the bile removed from the gall bladder in concentrations between 1:46 and 1:230 when none was present in the bile collected from the ductus choledochus 24 hours following intravenous administration of the dye in doses of 40 and 60 mgm. per kgm. of body weight.

Sodium salicylate or the sodium salt of diiodosalicylic acid injected intravenously in doses of 40 mgm. per kgm. of body weight did not appear in the bile in determinable quantities.

The excretion of iodine into the bile continues for 48 hours after the sodium salt of tetraiodophenolphthalein is given either intravenously or by mouth. It may sometimes be found in the gall bladder for as long as 96 hours.

BIBLIOGRAPHY

(1) HALPERT, B. AND M. T. HANKE. This Journal, 1929, lxxxviii, 351.
(2) GRAHAM, E. A., W. H. COLE, G. H. COPHER AND S. MOORE. Diseases of the gall bladder and bile ducts. Philadelphia, Lea and Febiger, 1928.
(3) YUDKIN, A. M. Trans. Amer. Ophthalmol. Soc., 1928, xxvi, 319.
(4) HALPERT, B. AND M. T. HANKE. Anat. Rec., 1929, xlii, 49.
(5) HALPERT, B., M. T. HANKE AND G. M. CURTIS. Journ. Clin. Invest., 1930, ix, 359.
(6) HALPERT, B. AND M. T. HANKE. This Journal, 1929, xc, 374.

THE ACTION OF CERTAIN DRUGS ON THE NICTITATING MEMBRANE

ARTURO ROSENBLUETH[1]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 19, 1932

During the study of the innervation of the nictitating membrane (n.m.) of the cat (Rosenblueth and Bard, 1932) one of the methods first used was that of examining the action of sympatho- and parasympathomimetic drugs on the organ. The results did not help to solve the problem then in view, in fact they appeared confusing and paradoxical when confronted with the data supplied by other methods. The action of the parasympathomimetic drugs on the n.m. is an exception to their usual effects. It was considered interesting to investigate further this paradoxical behavior.

Cats were used. The movements of the n.m. were recorded by the method described by Rosenblueth and Cannon (1932). The influence of previous denervation of the n.m. (right superior cervical ganglionectomy performed at least six days before) was studied, using the other membrane, acutely denervated, as a control. The contractions of both membranes were sometimes simultaneously recorded. Two levers of the same length and weight were adjusted to produce the same amplification and attached to corresponding points in the membranes. The head was fixed upright in a horizontal position and the levers were attached symmetrically.

Although the presence of the adrenals did not seem to affect the responses, these glands were removed or ligated *en masse* in all instances, to ensure a more steady preparation.

Dial anesthesia (between 0.6 cc. and 0.8 cc., intraperitoneally) was used throughout.

The drugs were injected into the femoral vein, through a glass canula. The rate was practically uniform (1 cc. per 5 seconds). The volume of each injection was usually 1 cc.

1. *Adrenalin.* The retracting action of adrenin on the n.m. has been long known. This action is sensitized (i.e., increased) by previous denervation and by cocaine (Rosenblueth and Cannon, loc. cit.). The sensitizing action of cocaine is more marked on the acutely denervated membrane.

[1] Fellow of the John Simon Guggenheim Memorial Foundation.

2. *Acetylcholine* causes a retraction of the n.m. (see figs. 1 and 2). This action is sensitized by previous denervation (see fig. 1) and by cocaine (see fig. 2). Atropine abolishes the effect of acetylcholine. The sensitization by cocaine is not as marked as the sensitization by this drug to adrenin.

3. *Pilocarpine* has the same action as acetylcholine. The responses are usually longer and irregular (i.e., the curve is not smooth). The action of pilocarpine had been previously reported by Dale and Laidlaw (1912).

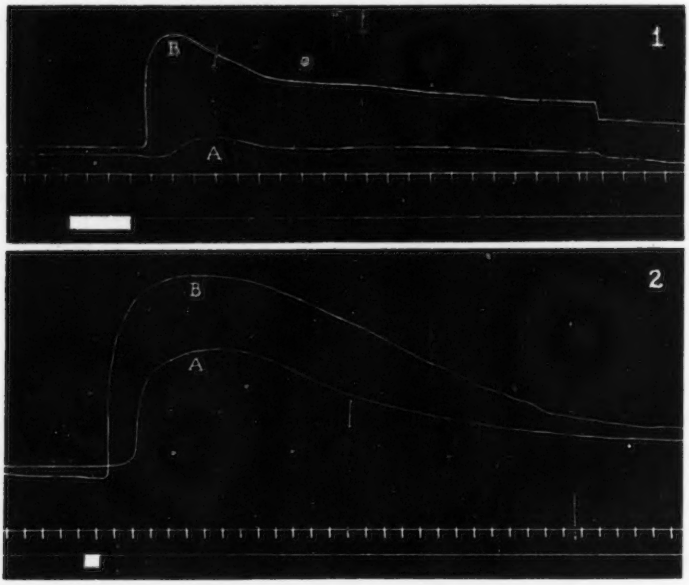


Fig. 1. Simultaneous record of the contraction of both nictitating membranes on injection of 0.5 cc. acetylcholine bromide, 1:100,000. Right n.m., *B*, denervated 6 days previously; left n.m., *A*, denervated acutely. Weight of the cat: 4.3 kgm. Dial: 0.7 cc. intraperitoneally.

Fig. 2. Contraction of the acutely denervated nictitating membrane on injection of 1 cc. acetylcholine bromide, 1:50,000, *A*, before, and *B*, after 23 mgm. cocaine. Weight of the cat: 3.1 kgm. Dial: 0.7 cc. per kilo intraperitoneally.

4. *Physostigmine* also has effects similar to those of acetylcholine. The responses are, however, usually comparatively small, and the sensitization less marked, especially when cocaine is used as a sensitizer. The response is likewise abolished by atropine.

5. *Atropine per se* has no action on the n.m.

6. *Histamine* contracts the n.m. The effects are increased by previous

denervation and by cocaine. Atropine in doses sufficient to antagonize the action of pilocarpine, acetylcholine and physostigmine (2 to 3 mgm. per kilo) does not abolish the response, although the effect may decrease slightly.

7. *Ergotoxine* (up to 9 mgm. per kilo) causes a permanent contraction of the n.m. The contraction is about the same in the acutely and the chronically denervated membranes. If adrenalin is then injected there appears a transitory slight relaxation. The same effects were observed by Dale (1906).

DISCUSSION. Since it is well established that the n.m. possesses a retractor smooth-muscle mechanism controlled by the sympathetic, the action of adrenin need not be commented upon.

The action of parasympathomimetic drugs may be explained in one of the following hypotheses:

a. We may be dealing with amphoteric drugs with marked predominance of action on the parasympathetic endings; when these are absent, the effect on the sympathetic endings would assert itself.

b. The sympathetic receptors of the n.m. may be exceptional in that they may be stimulated both by sympathomimetic and by parasympathomimetic drugs.

c. There may exist in different smooth muscle cells in the organism parasympathetic receptor mechanisms not connected with any nerves. This last hypothesis was suggested by Dale and Laidlaw (loc. cit.) and adopted by Hunt (1918) to explain the vasodilating effect of acetylcholine. This explanation seems improbable, however, for in the case of the n.m. the parasympathetic receptor would be acting in the same direction as does the sympathetic innervation of the muscle.

The first two hypotheses constitute really a single assumption: that the receptor mechanism connected normally with the sympathetic is susceptible of being acted upon by other substances than adrenin, including the parasympathomimetic drugs. That this view is probably correct is indicated by the fact that previous sympathetic denervation and cocaine, which typically sensitize to adrenin, also sensitize to the parasympathomimetic drugs, pilocarpine and acetylcholine. Sympathetic denervation should have no effect on parasympathetic receptors. As for cocaine, three experiments showed that it did not sensitize the action of acetylcholine and pilocarpine on the blood pressure.

The action of ergotoxine may be explained as a direct stimulation of the smooth muscle, similar to that exerted on the uterus.

The action of histamine appears similar to that of the parasympathomimetic drugs, being also affected by the sensitizers.

Physostigmine appears here as a parasympathomimetic drug, its effects being abolished by atropine.

SUMMARY

Acetylcholine, pilocarpine and physostigmine produce contraction of the smooth muscle in the nictitating membrane of the cat. This action is increased by previous denervation (see fig. 1) and by cocaine (see fig. 2), and is abolished by atropine.

Histamine has a similar effect, but atropine does not interfere with its action.

Ergotoxine causes a permanent contraction of the nictitating membrane and reverses the action of adrenin.

It is pointed out that the parasympathomimetic drugs act probably on the same receptor mechanism on which adrenin acts.

BIBLIOGRAPHY

DALE, H. H. 1906. Journ. Physiol., xxxix, 163.
DALE, H. H. AND P. P. LAIDLAW. 1912. Journ. Physiol., xlv, 1.
HUNT, R. 1918. This Journal, xlv, 231.
ROSENBLUETH, A. AND P. BARD. 1932. This Journal, in press.
ROSENBLUETH, A. AND W. B. CANNON. 1932. This Journal, xcix, 398.